

# THE PERITONEUM

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BYRON ROBINSON

HISTOLOGY AND PHYSIOLOGY





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THE PERITONEUM.





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BY

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CHICAGO, ILL.

AUTHOR OF "PRACTICAL INTESTINAL SURGERY," "LANDMARKS IN GYNÆCOLOGY," AND  
LIFE-SIZED CHART OF THE SYMPATHETIC NERVOUS SYSTEM.

PART I.

HISTOLOGY AND PHYSIOLOGY.

With 247 Illustrations.

*"To control a subject, to be its master, to concentrate upon it all that is absolutely necessary, demands in truth, the powers of a giant, and is more difficult than one would think."*—SCHILLER.

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THIS BOOK IS DEDICATED

TO

MY WIFE AND PROFESSIONAL ASSOCIATE, DR. LUCY WAITE

BY THE AUTHOR.





## PREFACE.

*The present volume is the outcome of a half-dozen years of personal labor in experiments in the peritoneum, in the study of its anatomy and in microscopical research. The labors of others have been consulted and credited. Every cut not credited has been drawn by myself from my own microscopical specimens.*

*Owing to an attempt to make each chapter as complete as possible, repetitions have been to a certain extent unavoidable. The book is a pioneer work, and every phase of opinion is represented in it. The author has advanced his own individual opinion as also the opinions of others.*

*The subject has been divided into the chapters presented with the hope of facilitating the study and comprehension of the histology and physiology of the peritoneum.*

*The chapter on technique is a short, imperfect sketch to simplify the work to beginners, as experience teaches that we do well what we do automatically. It is intended to publish in each volume as much bibliography as can be selected from the literature on the peritoneum. The bibliography contained in Volume I. is a careful selection from all sources.*

*My thanks are due to the late Dr. D. D. Bishop, whose untimely death we all deplore, for valuable suggestions in original work. Also to Miss Evangeline Sinclair for assistance in preparing the drawings for the engraver.*

BYRON ROBINSON,

Chicago, Ill.

August, 1897.





# CONTENTS.

## CHAPTER I.

HISTORICAL SKETCH.....	1
------------------------	---

## CHAPTER II.

THE HISTOLOGY AND PHYSIOLOGY OF THE PERITONEUM. Object of the Book—Fine Structure of the Peritoneum—Stomata Vera and Spuria—Planes of Tissue—Origin of the Serous Cavity by Pressure—Kinds of Animals used for Experiments—Silver Nitrate Staining—Methods of Staining and Study of the Endothelia—Preservation of the Peritoneum—The Significance of the Peritoneum—The Cell and its Establishment—Basement Membrane—Unsettled Points—Stomata—Interendothelial Space—Subjects for Discussion.....	14
--	----

## CHAPTER III.

THE ENDOTHELIA OF THE FREE PERITONEAL SURFACE. The Mesoblastic Origin of the Peritoneum—Ciliated Endothelia—The Term Endothelia—The Parts of an Endothelial Cell—The Views of His—The Endothelial Cell—Condition of the Interendothelial Space and Substance and its Physiology—Conclusions in Regard to the Interendothelial Space—What are the Functions of the Stomata Vera?—Stomata Spuria?—Shape of the Endothelia—The Arrangement of the Endothelia—The Influence of Blood Vessels on Endothelia—Regeneration of Endothelia—Germinating Endothelia—Anastomosis of Endothelial Cells—Conclusions in Regard to Peritoneal Endothelia.....	37
---	----

## CHAPTER IV.

SUPERITONEAL TISSUE. Kinds of Tissue and Cells—White Fibres—Elastic Fibres—Irregular Elements—Fixed Connective Tissue Cells—Planes of Tissue—Fat Globules—Diaphragm and Centrum Tendineum, Its Layers, Its Peritoneal Serosa—Stomata Vera—The Interendothelial Space—The Membrana Limitans—The Peritoneal Current—Perforations in the Membrana Limitans of the Diaphragm—The Centrum Tendineum is a bed of Lymphatics—Methods to Discover the Lymphatics—Valves on Lymph Trunks—The Diaphragm is the Special Locality for the Absorption of Solid Particles—The Lymphatics of the Diaphragm—Stomata—Lymph Nodes—Adipose Tissue—Fat—Pigment Cells of the Peritoneum—The Ground Substance of the Peritoneum—Germinating Patches and Tracts—Contents of the Subperitoneal Tissue—Conclusions in Regard to the Diaphragm—The Mesenteries are not Merely Duplicatures of the Peritoneum, but Primordial Structures.....	106
--	-----

## CHAPTER V.

THE BLOOD VESSELS OF THE PERITONEUM. Old View—The Essential Coats of Vessels—The Endothelia of Blood Vessels—Stomata of Blood Vessels—Action of Leucoeytes in Regard to Blood Vessels—Walls—Statements of Thoma—Study of the Endothelial Membrane—The Conditions for the Passage of Leucoeytes—Interendothelial Space—Conclusions in Regard to the Blood Vessels.....	177
---	-----

## CHAPTER VI.

THE LYMPHATICS OF THE PERITONEUM. Interstitial Spaces—Non-valved Capillaries—Valved Capillaries—Three Modes of Origin of Lymphatics—Imbibition—Osmosis—Filtration—Vital Processes—The Lymph Tubes and the Blood Tubes have a Common Ground in the Lymph Spaces—The Amphibian Lymph Saes—Stomata—Perivascular Space.....	204
---	-----

## CHAPTER VII.

NERVES OF THE PERITONEUM. History—Methods of Preparation—Medullated—Non-Medullated—Remak's Band—Vater-Paenian Corpuscle—Nerves of the Various Parts of the Peritoneum and of Different Animals, Especially the Cat—The Gold Chloride Method—Two Kinds of Medullated Nerves—Nerves of the Visceral and Parietal Peritoneum.....	255
--	-----

## CHAPTER VIII.

THE PHYSIOLOGY OF THE PERITONEUM. The Chronology of the Peritoneum—Stomata Vera—Stomata Spuria—Interendothelial Space—Blood Vessels—Lymph Vessels—Lymph Nodes—Nerves—Kinds of Cells—Methods of Origin of Peritoneum—Friction Pressure—Humidity—Polish—Serosity—Peritoneum has Distinct Structure and Function and Sensation—Interstitial Space—Similarity of Animals' Peritoneum in Structure and Function—Absorptive Capacity—Paths of Peritoneal Absorption—Methods of Absorption—Vital Cell Processes—Osmosis—Filtration—Imbibition—Intra-Abdominal Pressure—Author's Experiments and Observations on the Peritoneum—The Experiments of Adler and Metzler....	278
--	-----

## CHAPTER IX.

THE TECHNIQUE. Or Methods of the Preparations of Specimens of the Peritoneum for Microscopical Examinations.....	382
--	-----

## CHAPTER X.

A RESUME of the Physiology of the Peritoneum.....	393
---	-----

## ILLUSTRATIONS.

	PAGE.
Auerbach's Plexus.....	263
Blood Vessel.....	195, 198
Broad Ligament (human).....	155
(dog).....	235
Centrum Tendineum (human).....	157
(rabbit).....	133, 135, 141, 153, 171, 173, 219
Cisterna lymphatica magna .....	205, 213
septum .....	211
magna (frog).....	213, 219, 223, 232, 235, 237, 239, 241, 333
Capillary of (frog).....	288
of (dog's mesentery).....	336
Connective tissue fibrils.....	127
Diaphragm (human). .....	151, 159, 163, 165, 167
(dog).....	59, 155
(rabbit).....	83, 207, 216
.....	131
(hen).....	167
Epithelium (frog).....	45
Endothelium.....	49, 55, 57, 183
(of diaphragm).....	97
(dog).....	215, 229, 230
(small intestine of cat).....	83
(pleuro-costalis of cat).....	83
(craw-fish).....	91
Endothelia of small intestine (frog).....	282
of artery (dog).....	327
of thoracic duct (dog).....	327
of peritoneum (frog) .....	83
Fibres (elastic).....	125
Gall bladder (toad).....	69
(frog).....	273
Hepatic ligament (hen).....	77
Interendothelial canal (frog) .....	229, 230
Kidney (dog).....	333
Liver ligament (human)... ..	300
Ligamentum suspensorium hepatis .....	201
Lymphatics of intestines.....	336, 341
Lymph vessels.....	161, 233
Lymph capillaries (woodpecker).....	232, 246
sinus (dog) .....	245
sac (frog).....	250
Lymphatic duct.....	171
Mesenteric gland of guinea-pig.....	217
ox.....	139, 249
Mesogaster of mud turtle.....	95
Mesoduodenum (frog)....	25



Mesentery (human).....	16, 47, 189
(horse).....	61, 63, 93
(dog).....	113, 215, 285, 325
(rabbit).....	61, 89, 147
(cat).....	349
(frog).....	15, 71, 73, 75, 189, 225, 229, 237, 294
(frog's artery).....	10, 191, 193, 195, 197, 199
(leuciccus).....	319
(newt).....	325
Nerves.....	265
of blood vessel.....	275
of mesentery (frog).....	259
of peritoneum (cat).....	271, 275
of peritoneum.....	267
Omentum (human) ...	27, 33, 39, 50, 53, 55, 65, 112, 128, 145, 151, 159, 197, 199, 243
.....	245, 279, 294, 297, 306, 309
(ape).....	23, 51, 303, 333
(horse).....	35, 157
(sheep).....	28, 99, 300
(pig).....	27, 297, 319
(dog).....	31, 32, 69, 195, 239
(rabbit).....	20, 21, 29, 67, 137, 179, 185, 187, 237, 200, 209, 221, 217, 227, 291
(guinea-pig).....	17, 145
(gopher).....	65
(rat).....	163
(gastro-splenic of rabbit).....	79
(dog).....	101
(gastro-hepatic of dog).....	87
trabecula.....	124
Perivascular space (turtle).....	332
Pericardium (rabbit).....	20
Peritoneum (human).....	21, 303
(rabbit).....	41, 149
(turtle).....	49, 345
(frog).....	19, 22, 43, 136
(fish).....	67
(shy-poke).....	63
Peritoneal adhesions.....	167
Stomach (axolotl).....	311, 329
(turtle).....	311
(frog).....	311
Stomata (frog).....	282
Tissue, subserous (human).....	107, 109, 111, 118
elastic (human).....	119, 121, 123
Vater-Pacinian corpuscle.....	281, 275

## CHAPTER I.

### HISTORICAL SKETCH.

"That the historical information of matter diminishes with each generation of students is one of the worst phases of our present period of development in medicine. As a rule, it may be assumed that even the self-active younger worker's knowledge at the best covers from three to five years. Publications of five years past do not exist for them."—*R. Virchow, 1870.*

"Diligence and accuracy are the only merits which an historical writer may ascribe to himself."—*Gibbon's Rome.*

The earliest recorded observations of the lacteals (lymph vessels) in the peritoneum are those of Erasistratus, a Grecian physician, who was born about 340 B. C. and died 280 B. C. Erasistratus found the lacteals while examining the abdominal viscera of kids, but called them arteries. Thus, at least 2175 years ago the lacteals were observed in the peritoneum doing their silent work. Yet centuries came and went before science was able to interpret the phenomenon.

The above fact is related by Claudius Galenus (Galen) (A. D. 131–201 to 210), who lived in the then medical center of Pergamus and again at Rome. Galen must have been in possession of the writings of Erasistratus, for he noted the fact in regard to the lacteals of kids, 150 years after the death of Erasistratus. But this is not strange if one will study the medical history of Egypt as evolved in the stories of George Ebers, obtained from papyri of various dates; it will be noted that the Egyptians, in embalming the dead, eviscerated at least some of the bodies. Great establishments were maintained to do scientific embalming where bodies were eviscerated, allowing ample opportunity to observe many conditions of the viscera long before any nation allowed human dissections. No doubt Erasistratus visited Egypt and became acquainted with the human abdominal viscera through the embalmers. The "Ebers Papyrus," now the property of the Library of the University of Leipsic, Germany, contains quite a complete manual of Egyptian medicine of the 16th century, B. C., i. e., 3500 years ago. Unfortunately, in Egypt, the embalmers, known as paraschites, were considered unclean people, a despised class, really outcasts because they defiled or mutilated the sacred temple of the soul; hence, to a certain extent, physicians would not be likely to freely attend the work of the embalmers for fear of being defiled. But it is hardly possible that some of the magnificent Medical Faculty at Heliopolis did not

secretly or openly take advantage of the opportunities which the embalmers or paraschites could offer to physicians in studying the human viscera. It is reported that Egyptian kings, notably King Ptolemy, gave criminals to physicians to experiment on. Besides the Greeks and Egyptians performed experiments,—the only method to advance medical knowledge. Hence, it is very probable that the lacteals of the mesentery were many times observed by scientists among the Egyptians, Greeks and Romans, long before they were interpreted or recorded. Galen discovered the ganglia of the sympathetic nerves which supply the peritoneum so abundantly.

B. EUSTACHIUS (Italian anatomist, died 1574), saw the thoracic duct, but considered it a peculiar kind of vein, hence, up to the end of 1500, A. D., the functions of the lacteals situated in the mesentery were not recorded as interpreted. They were seen, but their office was unknown.

The beginning of the 16th century saw the dawn of peritoneal function as interpreted in the mesentery of dogs. The first idea of any function or structure of the peritoneum came from an experiment and was due to observing the lacteals which really only lie between its folds and though not absolutely separate from the peritoneum, are a secondary appendage of the peritoneum. The first physician to reap laurels from observations of peritoneal function and structure was the industrious and keen observer

GASPARO ASELLIO (Asellius) (1531–1626), an Italian professor of surgery and anatomy at Pavia, Italy. The principal discovery of his life was the lymphatic vessels of the mesentery. This he did at Pavia, July 23, 1623, by accident while performing vivisection on a dog shortly after the dog had eaten a meal. Three days later he demonstrated the same to his two celebrated friends, Dr. Alessandro Tadino (died 1661) and Dr. Settala (1552–1633). Asellio thought that the vasa lactea united in the pancreas and finally in the liver. He died at the age of 55 and his discovery was published by his friends. In the first editions of his work the cuts show the intestines natural size with much artistic effect. Asellio named these vessels lacteals from their carrying a milk-white fluid, a name they have ever since borne.

ASELLIO started physicians in the proper direction of experimentation to interpret nature's laws. The keen and active mind of a Swedish physician, Rudbeck, 28 years later made efficient and valuable use of the experimental method to advance the anatomy and physiology of the peritoneum.

FRANCIS GLISSON (1597–1671), an English anatomist of great distinction, the successor of Harvey in anatomy and surgery in the College of Physicians and Surgeons, concerned himself with the lymph

vessels. He taught, however, that the lymph was secreted by the nerves as well as the smallest arteries. He published and aided to spread Joyliffe's views of the lymph vessels in his "*Anatomia Hepatis*" (1654). Dr. Glisson, who wrote one year after Bartholin, supposed the lymph vessels arose from cavities and that their office was to absorb. Friedrich Hoffmann stated explicitly that the lymph vessels were to absorb. Glisson was the originator of the idea of "irritability," which later spread so wide in medical science.

THOMAS BARTHOLINUS (Bartholin) (1616-1680) was a Copenhagen anatomist, a Dane. He re-edited four editions of his father's (1585-1629) anatomy entitled "*Anatomia Nova*." Thomas began his studies of the lymph system in Leyden together with a study of Harvey's theory of the circulation. He pursued the studies at Padua and Naples. Bartholin discovered the thoracic duct in a man as Pecquet had discovered it in animals. He concerned himself especially for about ten years (1650 to 1661) with the lymph and chyle vessels, and enjoyed much fame and honor during life. Bartholin distinguished between chyle and lymph vessels, demonstrating many new views. Bartholin gave the pellucid tubes the name lymphatic vessels or *vasa lymphatica*. Later historians claim that Bartholin's admirers were over-zealous in attributing to him discoveries with peritoneal lymphatics which should belong to the Swede, Olaf Rudbeck.

JEAN PECQUET (1622-1674), a French anatomist, is famous for his discovery while still a student, in 1647, of the thoracic duct. He was an experimenter and was removing the heart from a dog when he observed that a milky fluid poured out of the vena cava superior. He first thought the fluid was pus, but further investigations taught him the true nature of the fluid. He now set to work to find how the lymph coursed from the heart to the intestines and was rewarded by the discovery of the thoracic duct. He published an essay on this subject in 1651 at Paris and an enlarged edition in 1655 and 1661. Pecquet died from an excessive drinking of brandy which he considered a panacea.

MARCELLO MALPIGHI (1628-1694) was an Italian anatomist and microscopist and made discoveries in almost all fields. His labors in the lymph system were published in 1697.

OLAF RUDBECK (1630-1702), a Swedish anatomist, first systematically made known Asellio's discovery of the lymphatics in 1651. Rudbeck was a noted investigator and an extensive experimenter, for in four years he sacrificed 400 different kinds of animals to demonstrate the lacteals and circulation just discovered by the Englishman, Harvey (1578-1657). Rudbeck independently discovered the cisterna chyli in 1651 and the thoracic duct in 1653. He noted the valves of the chyle vessels and the salty taste of the chyle. Rudbeck was famous in vast



experiment and in its interpretation; he was honored with a medalion, a bronze bust, and his name still lives in the plant species, "Rudbeckia." Curiously enough, like many other noted men, he was engaged in a long and bitter strife with his own university colleagues, on account of which he resigned from the Swedish University at Upsala. Rudbeck was a many-sided man, gifted, genial and talented, but he was reckless and domineering in his plans in regard to the college over which he presided, and as a result was resigned from his office. But his discoveries will last while time lasts. Rudbeck named the lymph vessels *duetus aquosi*, because they carried a fluid resembling water. History seems to be gradually according more and more the discoveries of the lymph vessels and lacteals to Rudbeck and less to Thomas Bartholin. It is thought that old writers attributed too much to Bartholin on account of his being in the famous Danish University. His discoveries in the lymphatic system were extensive from his methodical experiments. Rudbeck published an incredible number of books on widely different subjects.

GEORGE JOYLIFFE, F. R. C. P., (1637-1658), an English physician, was, together with Rudbeck and Bartholin, one of the discoverers of the lymph vessels. Joyliffe claims that he discovered the lymph vessels in the *vasa spermatica* in the spring of 1652, while he was acquiring the doctor's degree at Cambridge. Joyliffe had published nothing, but Glisson, to whom he had communicated his discovery, published it in 1654 in his "De Hepate." Later Dr. Timothy Clark (about 1664) published it more in detail in the London Physiological Transactions in the year 1668.

We are hence indebted to Asellio, to Rudbeck, to Joyliffe, to Bartholin and to Pecquet for the discovery of the lacteals, the general lymphatics, and the thoracic duct through a period of time from 1623 to 1702, or four score years. Bartholin and Hoffmann attributed to the lymph vessels the office of absorption. Lieberkuehn claimed that the origin of the lacteals were open mouths, hence, absorbents. Now, by analogy it was claimed that the other branch of the thoracic duct, viz.; the lymphatics, were also absorbents. Hence grew the knowledge that the physiology of the lymphatics of the peritoneum was that of absorption.

WESLING, 1634, saw the thoracic duct.

JOHN JAC SALZMANN (1679-1738), a Strasburg anatomist, injected the lymphatics with quicksilver.

JOHANN NATHANAEL LIEBERKUEHN (1711-1756) was a distinguished German anatomist. He was born and died in Berlin, Germany, where he did his chief labors, through his own invention, the solar microscope (1838). He was clever in producing artificial injections and making

elegant preparations, which are still preserved in the Berlin University. Lieberkuehn is reported to have discovered the mouths of the lacteals which stand open. From this supposed discovery it was once assumed from analogy that the other branch of the thoracic duct, the lymphatics, arose from cavities and that they were absorbents. He was a rare mechanical genius.

JOHANN FRIEDRICH MECKEL, the I or elder (1714–1774), was a noted anatomist of his age, a professor of anatomy, midwifery and botany, in Berlin, and grandfather of Meckel, the younger or II. Hewson (1739–1774) called Meckel “one of the best anatomists of this age,” and that he traced the lymphatic vessels into most parts of the body. Meckel wrote a work in 1772 entitled “*Nova Experimenta et Observationes de Finibres Venosum ac Vasorum Lymphaticorum in ductus Niscerague Excretoria Corporum Ejusque Structurae Utilitate*,” embodying his views of the lymphatics.

JOHN HUNTER (1728–1793), he who could toil so terribly, announced that there were organic pores in the wall of the peritoneum and blood vessels. The proposition of Hunter disturbed the writers of that day very considerably. But Hunter was a formidable opponent because he had experimented on over 3,000 animals. About one hundred years after Hunter announced that the peritoneum and blood vessels contained organized pores, Von Recklinghausen claimed he could see them with the microscope and still 30 years later Kolossow and Muscatello (1894) deny their existence, allowing history to repeat itself. The greatness of Hunter is seen in his vast conceptions of the designs of nature and his accurate observations of natural processes.

It is not certain how much William Hunter (1718–1783) contributed to this idea of organic pores, for he wrote an essay, “Remarks on the Cellular Membrane and Some of Its Diseases.” A century ago some writers spoke of the peritoneum as a cellular membrane.

ALEXANDER MONRO (Primus) (1697–1767).

ALEXANDER MONRO (Secundus) (1733–1817), father and son, did excellent work on the peritoneum, and the labor of the son is especially worthy of remembrance. One of the landmarks in the history of English medicine is the bitter and persistent quarrel between Hewson and Monro in regard to the discovery of the lymphatics of the peritoneum. Both doubtless made independent and valuable discoveries in the peritoneum, but history favors the most originality to Hewson.

ABRAHAM VATER (1684–1751), a German anatomist, was professor at Wittenburg. He discovered the corpuscle which bears his name. He also has a dilated portion of the gall ducts named after him, Vater’s diverticle.

WILLIAM HEWSON (1739–1774). Among the most philosophical in-

investigators in regard to the function and structure of the peritoneum was William Hewson, the one time partner of William Hunter. While dissecting before his class he received a wound and died from its effects. Thus perished at 35 one of the most promising of English investigators and experimenters. Our country received the services of Hewson's son, Thomas Ticknell Hewson (1772- ), President of the College of Physicians of Philadelphia.

Hewson was an untiring investigator and experimenter. He discovered the lymphatics in fishes, birds and amphibians. He was awarded a gold medal by the Royal Society of England for his valuable labors in the lymphatic system. He gave one of the most comprehensive descriptions of the lymphatic system up to his day and predicted stomata or organized pores about one hundred years before Recklinghausen discovered them by his unique experiments on the diaphragm of animals. In the fiery controversy of Hewson with Monro and the Hunters in regard to the priority of discovering the lymphatics, it appears to the writer that Hewson's name should bear the honor and that the English gold medal was justly awarded for valuable discoveries in the lymphatic system of man and animals.

Hewson's method of investigating was generally to ligate the thoracic duct or mesenteric artery before death or as soon after as possible, to obstruct the flow of lymph and distend the distal lymphatic vessels in the peritoneum, or to diligently labor until he could inject the lymphatics; e.g., he would ligate the vessels in a fish and then let the fish live as long as he could, whence his lymphatics would be widely distended. He made trips to the seashore to secure subjects upon which to experiment. Hewson was an original genius, a keen observer, a methodical investigator and a man of vast comprehensions. He was just and honest with his fellow-laborers, duly crediting them with the results of their discoveries.

WILLIAM CRUIKSHANK, (1745-1800), a Scotch anatomist, at Edinburgh was the assistant of William Hunter. His work entitled "Anatomy of the Absorbing Vessels of the Human Body," London, 1786, was translated in the French in 1787 and German in 1789. It was a valuable contribution to the lymphatics and passed through two English editions, the last 1790. Cruikshank's book enjoyed a wide confidence for many years and is extensively quoted by almost all writers on lymphatics.

It appears that the ancients knew little or nothing of the real nature of the general lymphatics, much less of the peritoneum. It is noted that Asellius reaped the first laurels in 1622, by discovering the lymphatics of the mesentery. This, of course, was an experiment, for Asellius opened a living dog and made the observation. He called the vessels lacteals, because they carried a milk-white fluid. Asellius en-



couraged further experiment, and twenty-nine years later (1651) Pecquet opened dogs and again saw the lacteals, but he pursued the study further and discovered the thoracic duct and followed it to the subclavian vein. Pecquet thus disproved the physiologic theory of his day, which was that the lacteals emptied into the liver. From 1651 to 1652 Rudbeck, Joyliffe and Thomas Bartholin discovered the other chief parts of the lymphatic system.

It appears that the mesenteric lymph vessels had been seen before these dates, but they went by other and erroneous names. Thus Erasistratus saw lacteals but called them arteries, as recorded by Galen. Eustachius saw the thoracic duct, but said it was a special kind of vein. Hence, up to 1652, Asellius, Rudbeck, Joyliffe and Bartholin deserve the credit of discovery and properly designating the place of lymphatic vessels. After this period Nuck added considerable knowledge to his methods of injecting the lymphatic glands, and Ruysch described the valves of the lymph vessels. The elder Meckel accurately described those known, but traced lymph vessels into many parts not previously known to possess them. In 1858 the Hunters (John Hunter, 1728-1793), (William Hunter, 1718-1783) and Monro (Alexander Monro, Jr., 1733-1817) raised a vehement controversy as to the office of the lymphatic vessels. The controversy was waged against the philosophic Hewson (1739-1774).

Dr. Glisson wrote in 1654 (F. Glisson, 1596-1677) that the vessels arose from cavities and that their office was to absorb. Friedrich Hoffmann called the lymphatic vessels an absorbent system. Haller opposed the view that the lymphatics were absorbents. From these dates onward the lymphatic system, especially of the peritoneum, rapidly attracted many laborers and investigators. The prominent names in history which have developed the lymphatic system from 1660 are quite numerous, but all were persistent experimenters and investigators of nature's laws.

William Hewson, who died at the age of thirty-five, gave some of the most comprehensive descriptions of the lymphatics, and predicted stomata. Almost one hundred years before Recklinghausen confirmed the discovery by experiment, Hewson showed that animals possessed a lymphatic system. For his valuable discoveries in the lymphatics, the Royal Society of England honored Hewson by a gold medal. The past one hundred years have brought the conclusions that the lymph fluid passes out of the blood vascular system into the lymph vascular system, and finally is returned to the blood vascular system; that the lymphatics of the peritoneum is a bed of nourishment and a drainage system. So far as function of the peritoneum was concerned and since structure was not known, function also remained unknown. The function and



structure of the peritoneum has only been learned by experimentation, and its present obscurity as regards structure and function will only be cleared by continued systematic experimentation after physiologic methods.

PAOLO MASCAGNI (1752–1815) was an Italian anatomist. He produced some epoch-making works in his publications over the lymph vessels of mammals. One work appeared in 1784, another in 1787, another in 1795. Mascagni's work demands a high appreciation from its thoroughness, breadth and originality. His method of injecting the lymph system has stood the test of time. It appears that many investigators are returning to the original results of Mascagni's experiments.

PHILIPPE PINEL (1755–1826), an eminent Paris physician, gave to Bichat his first impulse toward the study of the peritoneum from which such brilliant deductions arose.

THOMAS LAUTH (1758–1826), a famous Strasburg anatomist, who wrote in French, was a student of John Hunter and P. J. Desault (1744–1795) and successor of J. F. Lobstein (1736–1784) in the Strasburg chair of anatomy.

JOHN SHELDON (1763–1808), a distinguished English anatomist, the successor of William Hunter in the chair of anatomy to the Royal Academy of London. He published in 1784 "The History of the Absorbent System," a part of which was devoted to the lacteals.

MARIE FRANCOIS XAVIER BICHAT (1771–1802), a French anatomist and physiologist, a rarely gifted genius, was the eminent founder of the independent structure and function of the peritoneum. He established the fact that the peritoneum had an independent anatomy and an independent physiology, but Bichat was sufficiently honest to accord to the learned Pinel the honor of suggesting the view on which he labored with such important results. In fact, Bichat considered symptoms of disease unfruitful unless they accorded with pathologic anatomy. The fact that Pinel opposed this view led Bichat to study the peritoneum with the result of establishing forever its pathology, anatomy and physiology. Bichat was the founder of microscopical anatomy and pathology of the peritoneum. Pinel, the retired priest, and later profoundly eminent physician, was inclined to be theoretical. He wished to secure by the scientific cultivation of symptoms the diagnosis from symptoms alone. This very extreme of Pinel's stimulated Bichat to study the pathologic anatomy in the peritoneum.

This fruitful genius found ample field for his unbounded intellectual activity. Disease of the peritoneum must rest on physical facts—pathology. Thus, by close observation, Bichat established first that the peritoneum had a structure (anatomy) and a function (physiology) and was the first to call the peritoneum a serous membrane, a name it still

retains. When only about 29 years old he secured the appointment to the Hotel Dieu, where in six months he performed 600 autopsies. To show his zeal, he lived and slept in the autopsy room. Unfortunately he fell and struck his head, which was followed by what the French called "cephalic fever," and died at the age of 31. Thus perished one of the greatest geniuses, most comprehensive observers, and untiring workers of any age. His experiments have established the independence of tissue. Bichat published a work on the "*Traite des Membranes*," i. e., on the serous membranes, which shows his gifted powers at the best. It is a curious fact that though Bichat was an anatomist of the first rank it was purely the physicians who seized and set in practice his practical views. Later anatomists and pathologists perceived the valuable knowledge of Bichat.

KARL ASMUND RUDLOPHI (1771-1832) was a German anatomist, born at Stockholm, Norway, while his father held office there under the German government. He made some observations on the lymphatics in his "*Grundriss der Physiologie*" (1821), but since he was an opponent to vivisection, it must be held as speculative, as even some of his historians hint that he was of a speculative mind in some of his writings. However, being a keen observer, he is often quoted.

JOHANN FRIEDRICH MECKEL (1781-1833), the younger or second, the grandson, was professor of anatomy in Halle, filling the chair that his grandfather and father had filled. He was the most comprehensive anatomist of any age, and his labor stands the test of time. He published, with V. Fohman, some very valuable articles on the lymphatic system.

FRIEDRICH TIEDEMANN (1781-1861), was a German anatomist, filling a chair at Heidelberg. He was an untiring investigator. He was called to Heidelberg in 1816 as the first pure professor of anatomy and physiology. Henle succeeded Tiedemann in 1844 as an anatomist. Tiedemann had repeated calls to Bonn, Berlin, Giessen and Munchen. It appears that he devoted himself in later life to physiology, whence came his valuable work on the lymph vessels.

GILBERT BRECHET (1784-1845) was a French anatomist and the successor of Cruveilhier (1791-1845). He did some valuable work on the lymph vessels.

BARTOLOMEO PANIZZA (1785-1867), an Italian investigator and the friend of Mascagni, was professor of anatomy at Pavia 49 years. He discovered the lymph hearts or sacs of reptiles and a connection between the lymph sacs and the blood vessels, which is known as "foramen Panizzæ." He labored industriously in the lymph system with excellent reports. It appears that he coined the name *cisterna lymphatica*.

Magna. He published in 1833 a work entitled "*Sopra il Sistema Linfatico di Rettili.*" (About the lymphatic system of reptiles.)

VINCENT FOHMAN (1794–1837), was professor of anatomy in Lütich. He made extensive studies of the lymphatic system, laboring some with Meckel. In 1821 he published a work over the connection of the lymph vessels and veins; in 1826, the lymph system of the vertebrates, and in 1827 that of the fish was published. It appears that his theme of work for the remaining part of his life was the lymphatic system. He was a student of Tiedemann in Heidelberg for years, where his genius for observation was stimulated. His name is often quoted from his valuable and numerous essays in German and French on the lymphatics.

KARL FRIEDRICH THEODOR KRAUSE (1797–1868), a distinguished German anatomist at Hanover, produced some valuable reports on the lymphatic system, especially as to its origin.

ERNST FRIEDRICH GUSTAV HERBST (1803, alive in 1883) was born in Göttingen and was there a professor and wrote a work much quoted, "*Die Lymphgefässsystem und Seine Verrichtungen,*" 1844. ("The Lymph Vascular System and its Construction.")

FRIED GUSTAV JACOB HENLE (1809–1885) was one of the greatest of German anatomists. He had vast conceptions as well as accurate details of minute structure. His contribution to the function and structure of the peritoneum was especially valuable and far-reaching in its influence. He particularly brought forward the views of a fenestrated elastic membrane subjacent to the arterial endothelia. But to Henle must be ascribed the definite establishing of the cellular nature of the peritoneum. He demonstrated forever that the essential physiology and anatomy of the peritoneum is in its endothelia. Indifferent alike to opposition or laudation, Henle vigorously asserted and maintained this view through long years of indefatigable labor. His teachings have widely prevailed for half a century, and still exist. During his life and subsequently, Henle's labors have been very influential in directing histological investigations. Though a fertile discoverer, yet his value to mankind lies chiefly in establishing, through long and tedious labor, matters beyond doubt by unquestionable methods. From Henle's labors text-books assumed to publish his views are worthy of full confidence. Many of Henle's views may be obtained from his "*Handbuch der Systematischen Anatomie des Menschen,*" editions from 1841 to 1873. Perhaps in all medical history there has never existed a more persistent, indefatigable and industrious laborer than Henle.

GABRIEL GUSTAV VALENTIN (1810–1883) was born at Breslau—a celebrated physiologist of the highest rank. In 1834, in conjunction with his teacher (Johannes Evangelista Ritter Purkinje, 1787–1868), he



made the discovery of the movement of the cilia of the epithelia. He discovered the peritoneal endothelia, which was published in his *Reperitorium* (1836 to 1843).

MARIE PHILIBERT CONSTANT SAPPEY (1810, alive in 1883), an excellent French anatomist, wrote an excellent work on the anatomy, physiology and pathology of the lymphatics of man and vertebrates in 1873. He was the successor of Jaravey in Paris.

JOSEPH HYRTL (1811–1889), a professor of anatomy at Vienna, did excellent work in the lymph vessels of the abdominal viscera. He introduced the method of filling the lymph vessels with material to distend them by the "puncture method." Ludwig Teichmann honors Hyrtl as the one who taught him how to inject lymph vessels by the "einstich methode."

FILIPPO PACINNI (1812–1883) was an Italian physiologist who discovered a peculiar form of nerve ending in the peritoneum. It is called by some writers Pacinni's corpuscle.

SIR JOHN GOODSIR (1814–1883), a celebrated Scotch teacher of anatomy. He worked out the idea of cells and for his famous work Virchow dedicated to him his lectures on cellular pathology. He worked out views on the *membrana limitans*.

RUDOLF ALBERT KOELLIKER (1817, alive in 1883), a German physiologist and microscopist, professor at Wurzburg, produced excellent views on the lymphatics, and his labors induced many followers.

HUBERT LUSCHKA (1820 to 1875) was a renowned German anatomist, who wrote excellent works, especially "*Die Anatomie des Menschen*." In 1851, while occupying the Tübingen chair of anatomy, he wrote an essay on "*Die Structure der Serosen Haute des Menschen*." ("The Structure of the Serous Membranes of Man.") It contained about 100 pages and 3 illustrative tables. Luschka did not comprehend, even after Henle's vigorous assertions and tedious labor, that the endothelia is the essential element of the peritoneum. Luschka considered the essential element in the peritoneum to be its fibrous structures. Luschka's essay, which sums up the knowledge of his day (1851) in regard to the structure and function of the peritoneum, devotes only about three pages to the peritoneum. All the rest is dedicated to the serous membranes of the eye, nervous system, joints, ear and pleura. It is an excellent essay, the result of long personal labor, histologic, anatomic and pathologic, and contains the then prevailing views of the function and structure of the peritoneum. Luschka noted forty-five years ago that the constituent parts of the peritoneum were: 1, epithelia; 2, serous fibres; 3, cellular elements; 4, elastic fibres; 5, vessels and nerves.

HEINRICH FREY (1822, alive in 1883), a German physiologist of high

rank, did excellent work in the lymphatics of the peritoneum, some of which may be seen in his "Lehrbuch der Histologie und Histo Chemie," 1859.

LUDWIG TEICHMANN-STAWIARSKI TEICHMANN (1823, alive in 1883), a professor of anatomy at Krakau, wrote a very excellent work on the lymphatic system in 1861, which we were able to secure. It is a very valuable essay of about 100 pages and 18 beautiful copper plates. It is dedicated in deep gratitude to Joseph Hyrtl, the classical Vienna anatomist, who taught Teichmann how to study the lymphatics. It appears that Teichmann wrote the book from years of study and experiment.

BURDON-SANDERSON (1828, alive in 1896). In 1872 John Burdon-Sanderson, a professor of physiology in Oxford, England, produced, with E. Klein (living 1897), professor at the laboratory of the Brown Institute, London, one of the most excellent works on the peritoneum. To this work, "The Anatomy of the Lymphatic System of the Serous Membranes," published in 1873, I am indebted for the English presentation of the subject, especially for its accompanying cuts. Klein's labor in this book of 1873 is very commendable, and it forms one of the landmarks in the progress of the development of the function and structure of the peritoneum. In 1872 Klein and Burdon-Sanderson published the results of some excellent investigations in the *Centralblatt für Medizin, Wissensch.*, Nos. for January 2, 3, and 4, which are well worthy of perusal. In these long personal laboratory investigations and experiments, Klein brings forth his views of the three kinds of stomata. In these articles he claims that there are apertures, stomata vera, lined by polyhedral, granular, nucleated cells, that they are germinal endothelia. Another form of stomata vera is not lined by germinal endothelia, but simply the edge of surface endothelia, which aperture opens directly into the subjacent lymph channel. The third kind of stomata is the one in which it is supposed a connective tissue corpuscle juts upward between the endothelia. Klein also claimed that organized channels, stomata vera, produced a direct connection between the peritoneum and the subjacent lymph vessels.

WILHELM HIS (1830, alive in 1883), professor of anatomy at Leipsic, introduced the word endothelia in 1865 instead of epithelia, in his essay on "body cavity." It is a rare work, and in it His shows that endothelia of the peritoneum and the endothelia of the vessels are similar. His also advocates in this same essay that the peritoneal cavity is a lymph space or cleft.

FRIEDRICH DANIEL RECKLINGHAUSEN (1833, alive in 1896), professor of pathologic anatomy at Strasburg, is the distinguished German who discovered the use of nitrate of silver on the peritoneum and also the so-



called "wandering cell." Recklinghausen discovered the stomata on the diaphragm and that solid particles suspended in fluid would pass through the stomata in the diaphragm into the subjacent lymph vessels below. He used milk and other material, and his methods by which he discovered the stomata are unique and his persistence admirable. Recklinghausen demonstrated that Ag. NO<sub>3</sub> colored the interendothelial substance of the peritoneum dark brown.

JULIUS ARNOLD (1835, still alive), professor of pathology in Heidelberg, Germany, has labored extensively on the interendothelial substance of the peritoneum. He has demonstrated the method of capillary growth and the mechanism of the passage of the leucocytes through the peritoneal layer. His writings are chiefly found in Virchow's Archives.

GEORGE EDWARD RINDFLEISCH (1836, alive in 1883), Virchow's pupil, was an eminent pathologist.

GERBER (1850— ) and BRUNS (1841— ) advocated the cellular nature of the peritoneum.

The biography of authors extends to 1850 only.

The function and structure of the peritonenn was discovered by experiment.

The experiments proved the peritoneum to be a lymph-sac—an interstitial cleft or space.

Its function or physiology is to regulate fluid for nutrient and mechanical purposes. Its anatomical utility is chiefly mechanical, i. e., to allow limited action, to facilitate motion, to economize friction and conduct vessels and nerves to viscera.

The first step arrived at in the discovery of the function and structure of the peritoneum was the discovery of lacteals in a living dog by Asellio in 1623. The second step was the discovery of the thoracic duct by Pecquet in a dog in 1653. The third step was the combined discoveries of the stomata on animals, by Recklinghausen (1861) and the peritoneum being a lymph-bed, in the frog, by Ludwig (1866).

## CHAPTER II.

### THE HISTOLOGY AND PHYSIOLOGY OF THE PERITONEUM.

"Make choice of a subject beautiful and noble which shall afford ample field of matter wherein to expatiate."—*Dryden*.

The object of this volume is to present views, theoretical, practical and experimental, on the structure and function or the histology and physiology of the peritoneum. An attempt will be made to discuss the fine anatomical structure and to consider the complicated physiology. It is from a knowledge gained by observing structure through the microscope, and by the use of reagents and by studying function from experiments that we expect to secure methods to combat the invasion of disease into the peritoneum.

Peritonitis is one of the fell destroyers of the race, and he who brings forth means to check its fatal progress will be a benefactor to his fellows.

The peritoneal membrane is not dissimilar to the skin, being of about equal area. It presents a smooth surface covered by flat cells. By histology of the peritoneum, I mean the fine structure composing it, the elements entering into its structure which can only be observed by the aid of a microscope or a powerful lens. When one opens the abdomen of a healthy animal, the surface of the viscera and the internal abdominal wall present a shining appearance. The surface glistens to the eye and feels smooth to the fingers. The membrane is so transparent that many structures are visible beneath its surface. If a small piece of the peritoneum be snipped off with sharp scissors and placed in  $\frac{1}{4}$  per cent. solution of Ag. NO<sub>3</sub> for a few minutes and then mounted in a drop of glycerine, it will be observed under the microscope to have beautifully distinct dark lines separating various sized spaces from each other. The spaces are known as endothelia, and the dark colored lines are precipitated albuminous substance (space) lying between the edges of the endothelial cells, or plates. Thus the Ag. NO<sub>3</sub> solution and the microscope have dissolved the smooth shining serous membrane into distinct elements which make up the fine structure of the peritoneum. Besides the endothelial plates separated by dark sinuous lines, we can observe peculiar structures at the common junction of three or more plates, which are known as stomata (stigmata) vera, and also black dots situated along single interendothelial lines, which are known

as stomata spuria (pseudo-stomata). It may be we can see oval or round figures centrally or excentrically located in the endothelial plates, known as the nuclei. These endothelial plates are modified connective tissue corpuscles.

If we tear through the thin layer of endothelial plates, we come to a fine snow-white tissue. If this tissue be forcibly torn apart, it will resemble newly fallen snow. The corpuscles composing it may be likened



FIG. 1.—(Author.) Frog's mesentery drawn under very high power. (Oc. 4, ob. 8a R.). It shows sixteen endothelia, grouped about a stomata verum which contains six granular nucleated cells. Ag.  $\text{NO}_3$   $\frac{1}{2}$  per cent and mounted in glycerine. The figure is drawn as near to nature as possible. The stomata cells are intensely granular. Many stomata vera lie adjacent around which are grouped a less number of endothelia, but also only 3 to 5 granular cells exist in the stomatal rings. Notice, that between the endothelia there are distinctly two dark lines interrupted by cross lines of protoplasmic processes.

to the snow crystals. This is known as subserous tissue. Even with the naked eye the subserous tissue can be observed to be composed of fine, shiny, flat planes. The planes can be split and resplit into very fine sheets, which glisten and reflect light similar to the peritoneal surface. If a bit of this subserous tissue be mounted in glycerine and viewed with a microscope, its structure changes like magic in appearance. We behold innumerable fibres lying like fine silken bundles in

wavy lines parallel to each other. The fibres are of two kinds—the one known as white fibrous tissue, the other as the elastic fibres. Besides, many kinds of peculiar cells lie in this meshwork of fibrous and elastic tissue, such as wandering, vacuolated, branched and white blood cor-



FIG. 2—(Author.) Woman 45. Band of peritoneal adhesions stretched from uterus to rectum. 1, 1, 1, stomata vera; 2, 2, 2, stomata spuria; 3, 3, rift between endothelial cells, i. e., retraction of protoplasm. This is entirely new endothelium produced on the inflammatory exudate. In this inflammatory band the blood vessels and subserous tissue appear like the normal. Ag.  $\text{NO}_3$  applied.

pusecles (lymphoid cells). Again, in the meshes of this subserous tissue may be observed round, globular cells, known as fat cells. But the fat cells are only expanded connective tissue corpuscles. Thus the microscope has dissolved the mass of subserous tissue into white fibres, wandering and irregular cellular elements and fat globules. The above enumerated elements constitute the structure known as the peritoneum, or abdominal serosa, which will be the subject of our investigations.

The connective tissue corpuscles constitute with their process the bulk of the subserous tissue. In fact, it appears that the silken fibrous bundles are nothing else than the process of the connective tissue corpuscles or cell. The subserous tissue must be looked on as mesoblastic tissue which

contains and surrounds lymph vessels. In fact, the peritoneal sac is a mere lymph sac, or slit, in the mesoblastic tissue. By fluid pressure and motion independent of the epiblastic walls, the serous cavity arose and persisted. Every part and structure which the elements compose must be put to a microscopical test to learn, if possible, the vital functions of the great peritoneal sac. Elements must be understood to comprehend structure, and structure must be studied to appreciate its function, or physiology. A knowledge of the function, or physiology, of an organ places us in power to combat diseases which arise to menace the normal peritoneum. The methods which are best suited to study the elements



FIG. 3—(Author.) From frog's mesentery (Oc. 4, obj. 3, R). 1, stomum verum with a nucleated granular cell; 2, interendothelial stomata; 3, endothelium. Observe the grouping of cells around the stomum verum.



composing the structure known as the peritoneum are quite peculiar to itself. To secure useful results the structure and function must be studied together because, though a structure may be quite well comprehended under the microscope, its function may be almost entirely overlooked. With one structure in the peritoneum—stomata vera—this very puzzle of fitting structure and function together has not been solved. For the purpose of attempting to fit structure and function to

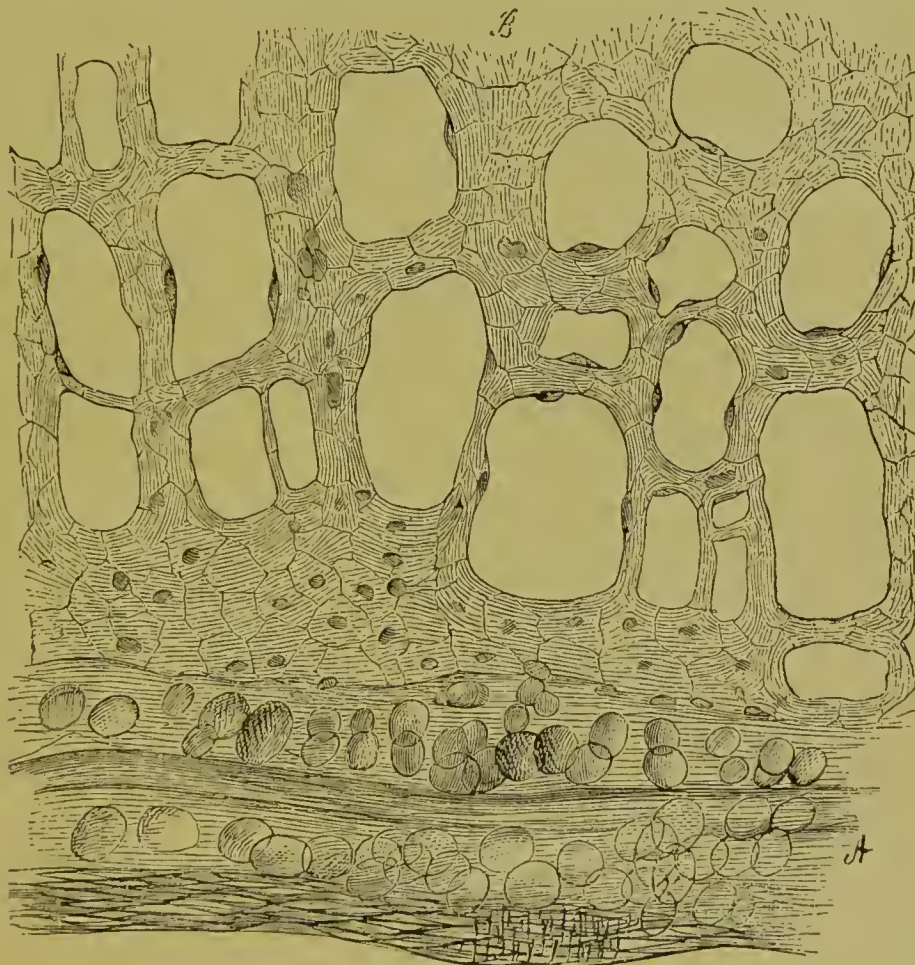


FIG. 4—(Handbook of the Phys. Lab. Vol. II, 1873.) Omentum of guinea-pig treated with silver. A. One of the principal trabeculae, containing blood vessels and fat cells. B. Fenestrated portion, the trabeculae of which are covered with flat endothelium. (Oc., 3; obj. 7. Tube of the microscope not drawn out.) This figure shows the fat cells, the stomata vera lying on the trabeculae which are so delicately covered by the endothelia.

a useful result, I have used many animals for examination and experimentation. I have made my observations on the peritoneum of the frog, rabbit, pig, horse, dog, hen, cow, sheep, toad, gopher, guinea-pig, shy-poke, rat, small birds, turtle and of man. The embryos of man and of some animals have been used also. The general method for microscopical study of the endothelium is to kill the animal, or to secure the peritoneum as soon as possible after death. In all the animals below



man it is best to stain the endothelia in situ. The reagent to employ is a solution of silver nitrate,  $\frac{1}{4}$  per cent. to  $\frac{1}{2}$  per cent., i. e., about two grains of  $\text{Ag. NO}_3$  to the ounce of distilled water. Distilled water must be used to dissolve the  $\text{Ag. NO}_3$  or it will combine with the many salts contained in common water and become precipitated. The  $\text{Ag. NO}_3$  should be prepared fresh every two weeks, or it seems to do inferior work.

The best endothelial staining may be secured with one-half per cent. of  $\text{Ag. NO}_3$  on the peritoneum, if it had lain very long in a dead animal; but with absolutely fresh peritoneum the most beautiful, distinct and delicate interendothelial lines were secured with one-quarter per cent. solution of  $\text{Ag. NO}_3$ . Again, the regulation of the intensity of



FIG. 5—(Author.) From gastro-splenic omentum of rabbit (Oc. 2. obj. 8a R.). It is stained with  $\text{Ag. NO}_3$ . 1, 10, 9, intraendothelial openings (stomata). 2, nucleus, clear in the center but well brown at its circumference, perhaps because it is elevated and is not bathed with fluid. 3, endothelium. 5, 5, 7, stomata vera. 6, has a nucleus in its granular cell. 4, stomata spuria. Note the grouping for the stomata vera.

light which falls on the silver stained peritoneum is just as significant as the strength of the  $\text{Ag. NO}_3$  solution. With one-quarter per cent  $\text{Ag. NO}_3$  solution in strong sunlight on fresh peritoneum, one minute may produce the finest and most beautiful endothelial lines, viewed with several hundred powers, and a lightly stained serosa can be retained. But if the sunlight be allowed to shine brightly and intensely on the specimen, one can gradually observe under the microscope the thickening and abrupt changes in the dark albuminate of silver lines. The dark line is due to the precipitation of fluid albumen by the silver salt. The employment of  $\text{Ag. NO}_3$  arose from the experiments of Von Recklinghausen in 1860. When the silver salt has remained on the peri-

toneal surface for several minutes it becomes of whitish gray color, and then under the action of sunlight soon turns dark brown. The part of the peritoneum which one desires to study after being stained in situ by gently pouring over it the silver solution should be cut out with sharp scissors and placed in a basin of distilled water for an hour, when it can be transferred to common water. Little pieces snipped off with very sharp scissors, so that no trauma may arise, and mounted in glycerine,



FIG. 6—(Author.) Sketched from visceral peritoneum of small intestine of frog. It was stained with silver nitrate and then stripped off. (Oc. 4, obj. 3, Reichert.) 1, 1, 1, 1, point to dots or interendothelial stomata on the surface of the endothelia; 2, 2, stomata spuria; 3, endothelia; 4, indicates a granular body, very likely a stoma verum with 3 nuclei. It appears beneath the endothelia, but the black dot at the junction of the four endothelial cells may be closed stoma verum which will further open as the granular germinating cells grow and multiply. 5 and 6, endothelia not drawn speckled or browned by the  $\text{Ag. NO}_3$ . Notice the irregularity and difference in the size of these endothelia compared with Fig. 1. The nuclei are not indicated.

suffice for the study of endothelia. Such glycerine specimens may be preserved for months, and they often improve with age. If the glycerine evaporates, renew it by allowing a drop to trickle under the cover-glass. It is necessary to study the endothelia in all grades and ages of silver staining to comprehend the amount and nature of the precipitated interendothelial substance, or that material which the  $\text{Ag. NO}_3$  solu-

tion precipitates and browns. Besides, all the various animals and embryos accessible should be employed to note comparisons.

It is a waste of time to attempt to solve the anatomic and physiologic study of the endothelia without  $\text{Ag. NO}_3$ . In regard to nucleus of endothelia, it is frequently brought out by the silver staining, but logwood staining from two to five minutes brings out a distinct, sharply outlined oval nucleus. In regard to subserous tissue, it needs no special preparation. Secure a piece from near the psoas muscle, or on



FIG. 7—Sketched from rabbit's omentum after it was penciled and stained with silver nitrate. They are, no doubt, germinal endothelia, as numerous similar endothelial patches remained after vigorous brushing with cotton on a toothpick. The endothelia are very much more brown than normal. The heavy dotted places on the endothelial surface is where the silver nitrate browned it strongly—perhaps adjacent to a nucleus. 1 points to the endothelial cell; 2, the excessive brown matter, perhaps adjacent to a nucleus; 3, is a black spot which may be a stomata spuria or where some matter has fallen out; 4, is either a stomata spuria in an obscure relation or perhaps a stomata interendothelium. This figure I sketched as near as possible to Nature (Oc. 4, obj. 3, Reichert). The endothelia are more round in this specimen than in the normal endothelia of the remainder of the omentum, indicating a young or germinating character. (Author.)

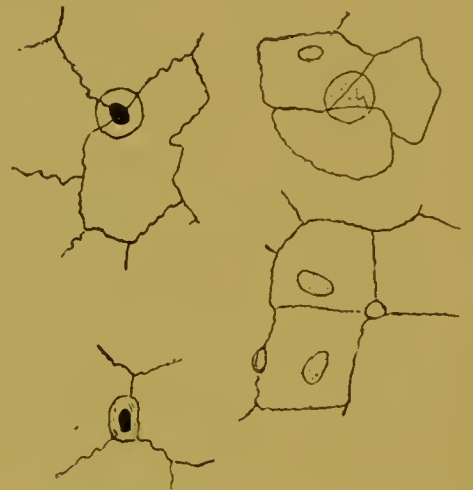


FIG. 8—(After Oedmansson, 1863.) In upper right hand corner is a silver staining of 3 endothelial cells, of the pericardium of a rabbit. One endothelial cell shows a nucleus. Below the surface of the common endothelia at the point of common junction of the three cells plates lies a round granular cell. Oedmansson does not indicate what it is, but we may designate this finely granular, round cell as a leucocyte attempting to gain the surface. At the lower right hand corner is a drawing of two endothelial plates of a rabbit's omentum. Each cell presents a nucleus.

the side of pelvis, where it is abundant and mount it in glycerine for study. If one wishes to see a confused mass of fibres, the tissues should be teased before mounting. However, if one wishes to study the lymphatic vessels of the peritoneum, the fresh peritoneal surface should be penciled or brushed with a little cotton on a toothpick, moistened with serous fluid. Brushing the surface 3 to 5 times one way is generally sufficient. The surface should then be stained with silver solution. The brushing abrades the surface of the peritoneum of its endothelia, and the endothelia of the lymphatic vessels are plainly visible. How-



ever, in some specimens the endothelia of the lymph vessels may be plainly observed through the transparent endothelia of the peritoneal surface. But it is not satisfactory, as the edges of the lymph vessels cannot be outlined satisfactorily.

For the preservation of the peritoneum 5 per cent., 10 per cent. to 20 per cent. of formaline in water is a good preservative. The typical animals on which to study the peritoneum are the frog and rabbit. They show about all variations and are convenient. The rabbit is especially good to demonstrate the lymphatics. The dog's lymphatics seem to be much more limited than the rabbit. The turtle's peritoneum is superior to all animals to study the lymphatics. In the turtle the lymphatics



FIG. 9—(After Oedmansson, 1863). The upper figure is a silvered specimen of a two days' old rabbit with many small and large openings between the cell-plates. The lower right corner shows a silvered specimen of endothelia of a rabbit's omentum. One can observe colored points on the interendothelial spaces only. Each plate shows a nucleus. Dr. Oedmansson remarks that the openings lead into a empty cell.



FIG. 10—Germinal endothelium from a band of peritoneal adhesions stretching from uterus to rectum. Women about 45. These endothelia appeared to be rapidly growing. Note 1 is torn from the bed 2 by the trauma of the pencil. Observe the numerous intra-endothelial stomata. Ag.  $\text{NO}_3$  applied. (Author.)

can be seen invaginated along the blood vessels, i. e., are excentric, while the blood vessels are concentric. The blood vessels may look like a dark rod in a clear white lymph vessel. For delicate work the embryo of the pig furnishes excellent material. The chief investigation of the peritoneum is generally done on the centrum tendineum of the diaphragm (pleural and abdominal side), on the omenta, on the mesenteric and visceral peritoneum. The diaphragm shows well lymph vessels and connective tissue corpuseles, while the omenta is the changing panorama of growth scenes, of vacuolation and germination. Nodules of germinating endothelia growing entirely above the common endothe-



lial surface, beautiful tracts of fat and lymph areas may be well observed on the omentum majus. Blood vessels are plainly visible, and if carefully stained, the vascular endothelium is beautifully apparent in elongated spindle-shape. It may be understood that the silver salts stain endothelia in all structures in the peritoneum, whether it be on the serous surface, lining blood vessels or enclosing lymph vessels. However, until one has exercised considerably in the use of silver salts,



FIG. 11—(Author.) Parietal peritoneum of frog (Oc. 2, obj. 8a, R.). Ag.  $\text{NO}_3$  applied but not penciled. No stomata are visible, but a short distance from this spot could be seen many stomata spuria. The nuclei are not indicated. 1, endothelium speckled brown by the Ag.  $\text{NO}_3$ . 2, endothelium not drawn speckled.

failures may be frequently expected, especially in staining lymph and blood vessel endothelia. Nerves require a special method to be made visible in the peritoneum. A careful technique and many months of patient labor are a requisite for even a superficial knowledge of the structure of the peritoneum, and especially to learn the function of the peritoneal endothelia. The practical results which come from any investigation of the peritoneum must be from a study of: (a) Its endo-

thelia, their structure and function; (b) the interendothelial space; (c) the stomata vera and spuria; (d) the origin, distribution and communication of the peritoneal lymph vessels; (e) the so-called membrana limitans, with its perforations; (f) the fibrous and elastic tissue; (g) the various cellular elements found on the subserous tissue; (h) the peritoneal nerves and (i) the blood vessels.

In the study of the function and structure of the peritoneum, in other words its anatomy and physiology, we have a wide field of action. Since it is probable that the peritoneal cavity is a lymph space or cleft, that it probably arose from fluid pressure and independent action of viscera and body wall, it may be observed that the lymph vessels of the peritoneum will be one of the essential features of its study. The peritoneum of all animals at command was studied microscopically under similar conditions with similar reagents. The peritoneum of embryos



FIG. 12—(Handbook of the Phys. Lab. Vol. II. 1873.) Fenestrated portion of omentum of an ape. Silver preparation of surface endothelium, showing the endothelium which covers a principal trabeculae (b). Here and there cells are seen which have germinative characters, and branched cells. (a). Meshwork of bundles of fibrous connective tissue. The stomata vera may be seen scattered over the whole length of the trabecula.

also was carefully studied. This work proved that the structure of the peritoneum of vertebrates and mammals is quite similar.

Again, I attempted to study the physiology of the peritoneum by the physiologic method of injecting colored matter, suspended in fluid, into the peritoneal cavity some time after which the animal was killed and the peritoneal membrane was put to a careful examination by the microscope. The use the peritoneum makes of the fine grains of coloring matter was a good criterion of its physiologic action. Experiments of this kind conducted in time too short to produce pathologic conditions on the endothelia illustrate the vast physiologic capacity of the peritoneum. Experimental physiology demonstrates that not all the peritoneum is endowed with like functions and powers, and it will finally lead to the better appreciation of its chief and essential functions. In the study of

peritoneal histology not only many different animals and their embryos are required, but modern microscopes from low power all the way to high-powered oil immersions are absolutely needed. Besides, one must have quite a number of reagents. Ag.  $\text{NO}_3$  is indispensable, yet its use requires considerable exercise to be constant or perfect. And light in the use of nitrate of silver is just as important as the salt itself. One cannot study long on the peritoneal endothelia before he becomes impressed that some reagent is required to fix cells and then to color when fixed. For the fixation process osmic acid is the best, and for coloring tannin and logwood serve an excellent purpose. I worked to disadvantage for some ten months on the peritoneal endothelia before I secured good fixation reagents to definitely set all the fine structures of the endothelial plate. It is not new to read that osmic acid is a fixation reagent for tissue, but it is said that its advantage in ordinary connective



FIG. 13—From frog's gall-bladder, stained with Ag.  $\text{NO}_3$ . 1, endothelium; 2, points to a stoma verum, with one black dot and three nuclei. There are apparently only two granular cells in the stoma verum, but that is on account, perhaps, of the closure of the mouth. (Author.)



FIG. 14—(Author) From mesoduodenum of frog (Oc. 4, obj. 8a, R.). It is stained with Ag.  $\text{NO}_3$ , and preserved in formaline. 1, endothelia speckled by Ag.  $\text{NO}_3$ ; 2, nucleus; 3, stoma verum with a granular cell fallen out; 4, granular cell lining stoma verum; 5, black body; 6, endothelium, not speckled.

tissue is not so much superior as to delay long over it. Dr. Kolossov of Moscow, Russia, has written an article on the pleuro-peritoneal endothelia in which he combines the reagents, osmic acid and tannin or pyrogallic acid. This combined reagent with Ag.  $\text{NO}_3$  produced in my hands such superior results that much of my investigation was reviewed. Osmic acid, tannin and Ag.  $\text{NO}_3$  with a 1-15 oil immersion lens is requisite to do effective work with the peritoneal endothelia. With such instruments one can secure brilliant pictures for study and unlimited fields for interpretation of structure and function. The best microscopic slides of the above reagents are obtained from perfectly fresh peritoneum, better used while the peritoneum is still warm from the newly killed animal. The gross or microscopic anatomist announces the peritoneum to be a closed sac except in the female, where it is perforated



by two apertures for the oviducts to transport the deshisced ovum, which projects into the peritoneal sac, into the mucosa of the genitals. But the microscope with reagents appears to teach that the peritoneum is not only perforated by two large microscopic apertures, but by thousands of microscopic apertures which connect the peritoneum directly with large and wide subjacent lymph channels. The peritoneum stands in intimate connection and vital relations with the physiology of the whole

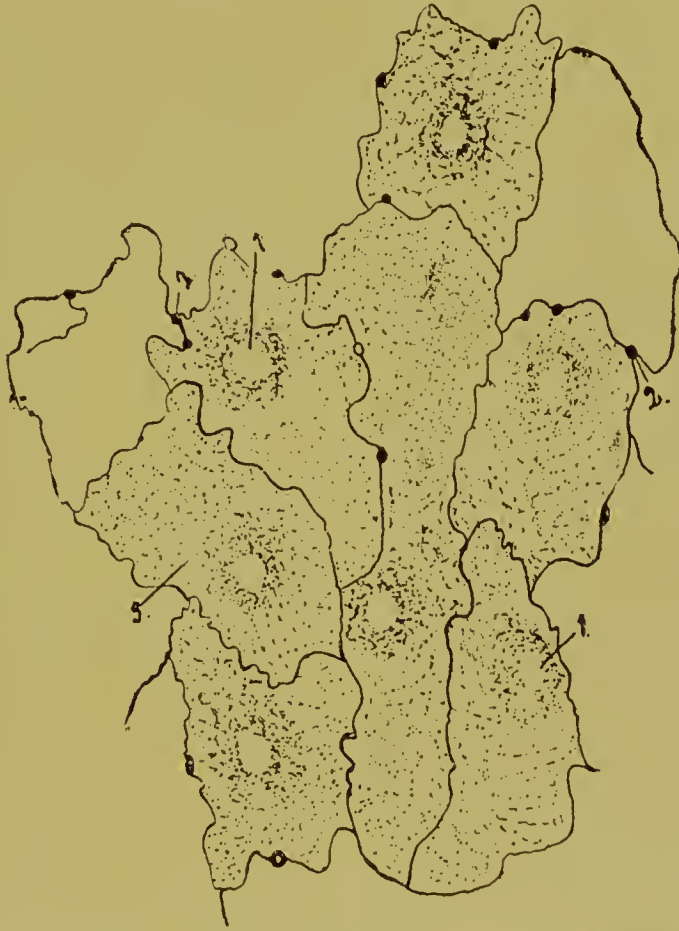


FIG. 15—(Author.) Represents the endothelium covering the lymphatic vessels of a frog's mesoduodenum. The mesoduodenum was brushed with a little cotton on a toothpick for four to six times, which cleared away the peritoneal endothelium. 1, nueleus; 2, 2, stomata spuria; 3, endothelium. Notice how sinuous the interendothelial lines are, which is characteristic of endothelia covering lymph vessels. The nuelei are generally situated eccentrically. No stomata vera exist in this specimen, which is rare.

system. It lies on the borderland of vast structures and laboratories of assimilation. It is a great mediator of fluid interchanges, and it holds the key of life and death in the battle against infectious invasions. The peritoneum has within its embrace the citadel of all life's nutritive organs, and it protects them with a jealous care against all microbic invasions. It is the great protector of the assimilation organs, and assumes equally protective care over the genito-urinary apparatus.



It will not seem strange to state that the organ known as the peritoneum is composed of simple cells when one recognizes the penetrating power of the microscope and the vigorous and far-reaching investigations of the nineteenth century. To understand the whole structure of the peritoneum, we must analyze every element and its relations to all other elements. Some of the chief cells of the peritoneum will be discussed while the other cells will be merely mentioned.

It was a long and difficult struggle to establish the individuality and independence of the cell. Schleiden had made careful and extensive studies of vegetable cells, and at this time all cells were considered to possess (a) a cell wall, (b) cell contents, and (c) a nucleus. Schwann studied the tissues of the frog and found that the frog had a cell and a nucleus also, and this induced him to compare animal and vegetable cells. The analogous structure and similarity of function brought out by Schwann made his labor of great worth. Muller popularized Schwann's works. Valentin discovered the nucleus of the cells of the epidermis, and he compared their similarity to plant cells. At this time, it must be remembered, it was thought that almost everything depended on the vessels and that the cells were not independent, but dependent on the vessels. Henle made a break in this train of thought when he showed that the epidermic cells became larger in diameter as they approached the surface—thus demonstrating that the increase was not dependent on vessels. But Schwann was a great generalizer, and he employed all the discoveries of Schleiden in the vegetable kingdom, and compared them with his own labors in animal tissue, and he finally announced that vegetable and animal cells are completely analogous and just as independent in mode of growth. He added the important remark that vessels only cause variation in the distribution of nutrition of the body and that cells do not depend on vessels. Johannes Miller adopted Schwann's views, and thus popularized his labors, incidentally remarking that Schwann's works were the most remarkable that had appeared in histology. At this period of the world appeared the immortal Bichat, whom the French claim founded histology by employing the discoveries of Schleiden in the plant cells and those of Schwann in the animal cells. Bichat was the pioneer who first showed the distinct anatomical independence of the peritoneum, and hence its capacity of being attacked by distinct diseases. The most magnificent phenomenon of this century, Virchow, was just appearing in the field of science, and he quickly generalized the whole organism as being a free state, and the cells as individuals endowed with equal privileges and equal powers. Thus by slow and almost inscrutable increments, the whole physiologic significance of the cell was modified. At this period the industrious Max Schultze had defined a cell as a clump of matter with a nucleus.

Remak had introduced the word protoplasm, for animal cells. Max Schultze, however, demonstrated that cells were not merely vesicular, but the protoplasm of a cell might be fluid, semi-solid or solid. So far, then, we have Max Schultze's definition of a cell to be a mass of protoplasm with a nucleus. Leydig said a membrane was not necessary to a cell. Finally, the last break in traditional thought was made by the celebrated Brecke, who stated that a nucleus was not necessary to any cell. We now have the final definition of a cell, that is, a mass of protoplasm. Every step in establishing a cell on an independent basis was firmly



FIG. 16—(Author.) Omentum majus of a new-born child, who died immediately after a normal labor. (Oc. 2, ob. 8a.) It does not show fenestration at point of examination of specimen. 1, stoma verum lined with large, granular polyhedral cells. 2 points to an endothelial cell which seems to share in the stoma verum. It has in it four clumps of debris. Ag.  $\text{NO}_3$  applied. 2 and 4 show quite irregular endothelia surrounding the stoma verum. Some of the growth processes of the stomata vera appear to be going on under the endothelia and it shimmers through the transparent endothelia. Much might be said in regard to the stoma verum being a soft, protoplasmic mass, and corpuscles (blood and lymph) could have ingress and egress leaving no mark.



FIG. 17—(Author.) Pig's gastro-splenic omentum. (Oc. 2, ob. 8a R.). Ag.  $\text{NO}_3$  applied. It lies over a field of fat, hence irregular endothelia from irregular and rapid growth of fat. Stomata vera are very numerous on this specimen. 1, stoma verum. It is divided representing two germinal endothelia. 2, 2, 2, stomata spuria: 3 appears to have a stoma verum broken open with a part of the germinating wall floating to one side. 5, a closed stoma verum; 6, intraendothelial stomata.

contested. The further work on cells consisted chiefly in defining their peculiarities, such as how they grow, how they move and reproduce. Virchow announced that the ciliated movement must be due to an elastic property of the cell structure. Stricker noted that substances found in the cell might come from without or be a chemical product of protoplasm of the cell. Haeckel also worked out similar views.

Celebrated names may be noted along the historic lines of work announcing the cellular nature of plants and animals, as Leeuwenhoek, Grew, Hooke, Mirbel, and Turpin, and with the improvement of the

microscope came entirely new views. In 1840 Purkinje applied the term protoplasm to the substance of the animal cells. But the general use of the name protoplasm was due to its employment by Mohl in 1846. Mohl applied the term protoplasm to both animal and plant cell substance. In 1835, however, Dujardin applied the term sarcode to the same substance that Purkinje and Mohl called protoplasm. It was thought up to thirty years ago that a cell or protoplasm was homogeneous and structureless, but Klein, Heitzmann and Frommann showed that a cell is reticulated.

A cell is a mass of protoplasm which exhibits the properties of living animals, i. e., it eats, grows and breeds. In other words, a cell assim-

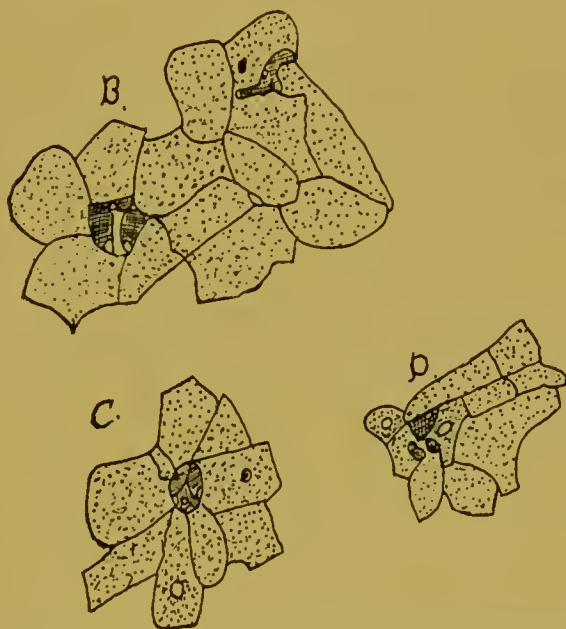


FIG. 18—(Author.) Sheep's omentum majus. (Oc. 4, ob. 8a.) The figures show grouping around stomata vera. A, B, C, shows the endothelia grouped about them.

lates, increases in size and reproduces its kind. The establishment of a cell as an independent organism leads the way to the definition of the different kinds of cells with their different properties, structure and functions. It also paved the way for Virchow to found cellular pathology. Cells are modified into connective tissue, muscular and nerve tissue; into blood, bone, fat and gland. Our study will be confined to those cells which belong directly to the peritoneum, such as the endothelium, the connective tissue, muscular, elastic, glandular

tissue with nerve tissue. The peritoneum consists of:

1.—Connective tissue cell so modified that it presents a smooth surface and called an endothelial plate. It is smooth on the free side and rough on the attached or opposite side. The free surface is generally highest in the middle, and perhaps this is the reason the nucleus shows a white oval, as its summit was not covered by albuminous fluid which Ag.  $\text{NO}_3$  precipitates or blackens and browns. The whole cell presents an oval form, as is easily seen when the endothelial cell is brushed from its bed. It leaves an oval depression in the ground substance. Carl Ludwig's school, Muscatello and others appear to think that the original border of the endothelial cell is polygonal, but as to this assertion, after examining hundreds of specimens from adult animals and embryos, I am in doubt. It appears from my examinations that the



endothelia are subject to much change by growth processes. It may appear strange that the endothelia should be considered as connective cells, but by careful study in brushing the endothelia of the peritoneal ground substance, many fine, irregular processes may be observed projecting from the non-free surface of the endothelial plates. These projecting processes of connective cells, so that the only modification occurring in the connective tissue cell to make it an endothelial plate is to flatten it on one side by continual friction. In the lower animals the projecting processes of the attached surface of the endothelial plate are quite apparent. The endothelial cell, or better, plate, has an oval or round nucleus which can easily be stained with logwood. The nucleus of the endothelial cell may be double or it may be centrally or eccentrically located. The nucleus may possess nucleoli. The endothelial plates possess a varied degree of transparency. Some are quite opaque and others are so transparent that underlying structures in strong light may be seen. The endothelial plate is very elastic, as I have often observed; it will spring backward and forward by waving fluid under the microscope, and clinically this is known by the distension and contraction of the peritoneal endothelia in ascites.

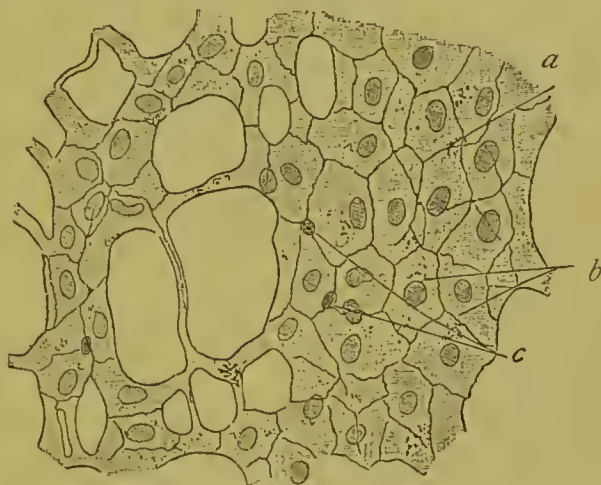


FIG. 19—(After Stohr, 1894.) A silvered piece of omentum majus of rabbit 240 times magnified. Thick and thin connective tissue bundles form meshes. At a, the endothelial cells of the opposite side are shimmering through: b, endothelial cells: c, nucleus from connective tissue cells. This last is Stohr's individual interpretation which is different than the general rule, for c is generally interpreted as stomata spuria and vera.

In this work we discard the term interendothelial substance and substitute for it interendothelial space. We also adopt the views of Drs. Kolossow and Ranvier in dividing the endothelial cell into a cover-plate, i. e., an indurated, metamorphized portion of the protoplasm and the protoplasmic portion of the cell containing the nucleus. The endothelial cells, like connective tissue cells, according to old authors, are held together at the edges by an albuminous semi-fluid substance so as to produce an unbroken membrane, and no doubt the physiology of the peritoneum must be looked for in the intercellular or interendothelial space. But today we say the endothelia are also held together by anastomotic processes made manifest by Ag.  $\text{NO}_3$ , but their anastomotic processes lie below the cover-plate. Kolossow asserts that fine cilia may be found on the endothelial surface,



but I have so far not been able every time to confirm his view. Klein claims and produces cuts to the effect that the endothelial possess a fine meshwork of fibres in its interior. The processes of the endothelial plate run in different directions. The intercellular network of Klein requires a high power to differentiate. In general the endothelium are so transparent that the only practical method to study them is to color them with Ag. NO<sub>3</sub> solution. Logwood produces a clear oval or round nucleus, but the nucleus is often plainly observed without the use of reagents. Certain peculiar openings or deposits occur on the plates which I have termed interendothelial stomata. Acetic acid makes the endothelial swell, while alcohol and formaline contract it. Muller's fluid preserves it in the most natural state. Further remarks will be made on the endothelia in another portion of this work.

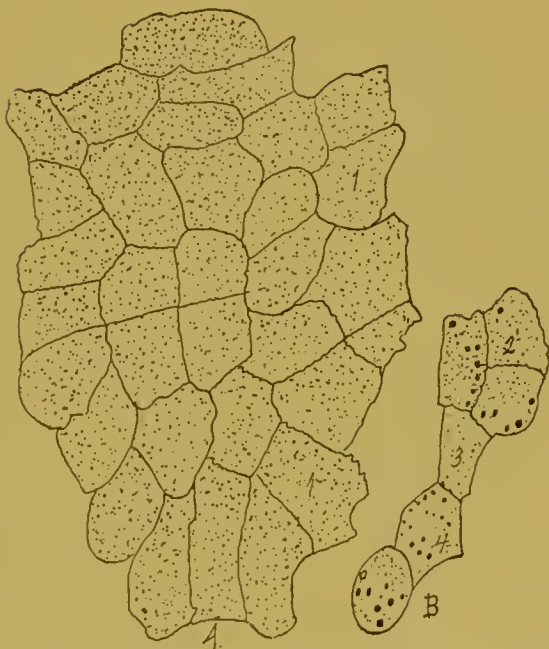


FIG. 20—A dog's mesentery (Oc. 2, ob. 8a R.). No signs of stomata of any kind exist. The endothelial plates are very brown and granular and lie on the border of a field so granular as to look like a tract of germinal endothelial. B, 2 and 4 endothelia marked with dots on are taken closely to the other, 3, no dots on. In B the intercellular lines under high power are continuous dots. (Author.)

2.—We will consider a basic substance on which the peritoneal endothelia rest. It is the basement membrane of Todd and Bowman (1847), the membrana limitans of Bizzozero (1873). The membrane had been claimed to exist and not to exist by a number of highly respected investigators. It is a clear, glass-like, fibreless substance lying immediately under the endothelia. As a distinct membrane it is difficult to isolate satisfactorily and be sure

of its nature. But this glass-like membrane is easily observed, but not easy to isolate. The peculiarity of this membrana limitans, according to Bizzozero, is that it is perforated by round or oval openings on the centrum tendineum of the diaphragm and that these apertures lend to the diaphragm the traditional power of absorption above all other portions of the abdominal serosa. Also that the membrana limitans gives the explanation to an experiment introduced by Bichat and confirmed by Ranvier and Muscatello, where if the mesenterium be blown up with air it will long remain distended, separating the mesenterium into a mesenterii membrana propria, the neuro-vascular visceral pedicle

and two surface layers, viz.: the endothelia supported on and by the membrana limitans, on each side of the mesenterium. The membrana limitans not being perforated, except on the diaphragmatic serosa, it does not allow the air to escape. The most perfect example which I secured of the membrana limitans, showing its perforations in the diaphragm, was from the body of a female fourteen years old; dogs furnish good examples, also.

3.—The connective tissue cells. These are numerous in the subserous



FIG. 21.—(Author.) Young dog's gastro-splenic omentum, 3 months old. 1, rift in between granular cells and endothelium. (Ob. 2.) This figure is difficult to interpret. It is drawn very carefully to nature. It is drawn on a non-fenestrating trabecula of which three is the edge. 1 and 1 appear to me to represent the stomata vera contracted from the plate's edge. 2, 2, 2, 2, represent still further stomata vera; 2, nucleus. The loop 4 doubtless represents a closed stomata vera at 5 and a precipitate at 6, yet such rifts as at 6 are usually reddish brown, granular young cells. 7, 7, 7, doubtless represent stomata vera with guard cells fallen out; 8, 8, stomata spuria; 9, 9, endothelia. Note the irregularity of all elements of endothelia, stomata and even precipitates.

tissue, and it is supposed by many that the fibrous tissue is derived from the elongated processes of the connective tissue cells. Perhaps the most typical specimen to observe a connective tissue corpuscle is in the blood vessel wall of the broad ligament of a gestating turtle. In it the deposit of pigment makes the outline of the cell prominent in all its ramifications. The elastic fibres may be formed in other ways according to Ranvier, i. e., by fusion of small globules.

4.—Wandering cell (white blood corpuscles), which are very important elements in both health and disease of the peritoneum; 5.—Branched cell; 6.—Vacuolated cell; 7.—Pigment cell; 8.—Fat cell, which is only connective tissue cells expanded by accumulated oil globules; 9.—

Muscle cell; 10.—Nerve cell; 11.—Vascular cells (mesenteric glands); 13.—The interendothelial space; 14.—Stomata vera (cells); 15.—Stomata spuria (connective tissue corpuscles); 16.—The elastic tissue cell.

The elastic cell is what gives to the peritoneum its peculiar quality of adaptation to environments. The elastic cell must belong to a certain extent to the endothelia for which they are capable of extension and contraction to a wide degree. The elastic fibre, composed of course of

cells, is produced, according to Ranvier, by fusion of small globules. The elastic cell is very abundant; it is associated with the genital organs and endows them with the wonderful power of changing their conditions and of returning to normal without loss of integrity. In many animals as rabbit, cat, dog, horse, man, etc., the mesenterial supports are a meshwork of elastic fibres. The elastic cells are the elements which allow accommodations of adaptations of adjustment of the peritoneum to surroundings and circumstances. They prevent the genitals from fatal and inevitable prolapse. They keep tissues in compact state.

In regard to the unsettled points in the histology and physiology of the peritoneum, I shall attempt to give views held by scientific observers. The difference of opinion as to structure and function of the peritoneum is considerable. No doubt the variation is due largely to different conditions of investigations, different experiments on different animals, different microscopical technique as well as difference in the reagents employed, microscopical experience and capacity of observation and power of interpretation. But after allowing for personal equations and variations of conditions, there still remain very essential and irreconcilable views for which to account. The view which holds that there exist preformed openings between the endothelial cells and the opposite view are not reconcilable. Respected observers exist on both sides. It is claimed by some and denied by others that the lymphatic vessels stand in direct open communication with the peritoneal cavity. Again, one class of observers holds the so-called stomata vera of the peritoneum as centers of formation for new endothelial cells to supply the place of dying comrades, while the others consider them as organized vertical channels connecting the peritoneal cavity and the subjacent lymph vessels. These vital differences can only be settled by continued experiments and observations. The author's microscopical labors on the peritoneum have continued steadily for two years, and like others, he has taken his position in this variation of opinion. In the following labors on the histology and physiology of the peritoneum, it will be noticed that distinct portions of the peritoneum are finally selected for typical work. These typical localities are the diaphragm, the tunica vaginalis, the omenta and mesenterium. The author is finally convinced that more is to be gained by continued labor and interpretation of the peritoneum of a few animals than the examination of many animals. What we need is more perfect technique and more facts to support interpretations.

The diaphragm is selected because the typical stomata and lymph vessels may there be seen. By gently brushing the fresh pleural surface off the diaphragm with a little cotton wound on a toothpick and wet in



the serum of the peritoneum and staining with  $\text{Ag. NO}_3$ ,  $\frac{1}{2}$  per cent., the lymph vessels may be seen in the most beautiful type. The omenta is used because it requires no preparation except snipping off, staining and mounting in glycerine to show a beautiful panorama of structures. The mesenteries may be as conveniently employed, but do not show structures so typically. All trauma or dragging on the peritoneum destroys quickly its relations, for the interendothelial space is easily disturbed, stretched or compressed. Even the forces of blood currents in a vessel alter the shape and direction of the endothelia.

The blood vessels are easily studied in the peritoneum, but the nerves and their endings consume much labor and time. The subserous tissue, muscle, fibrous and elastic tissues require no special technique. It is commended in investigations of peritoneum that the selection of two animals for continued observations is most profitable. The frog and rabbit are two typical animals and easily obtained. By persevering with two conveniently accessible animals more accuracy may be obtained, as there is less danger of being confused by the slight peculiar variations in many animals. The views of individual observers of the peritoneum will be discussed in the appropriate place.

We assume throughout this work a protoplasmic connection of the endothelial cells and not an interendothelial substance. It is the application of a suggestion of Heitzmann and Pflueger that all cells are organically bound together in colonies. The endothelia are no exception to the rule. Again, it will be noted that the chief bulk of work is involved with the function and structure of the endothelial membrane composing the blood vascular, lymph vascular and peritoneal endothelia.



FIG. 22—(Author.) Omentum of a woman of thirty. Dead 24 hours, from eclampsia with no peritonitis. (Oc. 4, ob. 3.) 1 and 2 point to two very brown granular polyhedral masses (cells). It appears to be a typical stoma verum. Two nuclei exist in the stoma verum cells of which three indicates one. 4, 5, points to another distinct stoma verum. Note the grouping around both: 4, endothelia; 6, 7, edge of trabecula; 8, stoma verum; 9, stoma spirum; 10, rift in cell. Note irregularity of endothelial both in size and grouping.  $\text{Ag. NO}_3$ ,  $\frac{1}{2}$  per cent.



The chief function and structure of the peritoneum is involved in this endothelial membrane with its interendothelial space. With the plates of the endothelial membranes, i. e., the blood and lymph vascular and peritoneal endothelia, we have but little to do. It is almost entirely with the interendothelial space that our labors are engaged. In the absorption of peritoneal fluids, however, the endothelial cover-plate may play a considerable role. We discard the hypothetic cement substance and replace it by the term interendothelial space. In this space may be observed the anastomotic protoplasmic processes which bind the endothelia together into colonies, besides the structures *stigmata*, the exact nature of which still remains in the fields of unsettled opinion. The peritoneal cavity is the receptacle for white blood corpuscles which not only nourish and replenish its endothelia, but protect the cavity from foreign invasions. We learn from long experimental labors that a current exists in the abdominal cavity toward the diaphragm, that the diaphragm is a vast bed of lymphatics and possessed of extensive absorptive powers.

In this work we have examined the peritoneum of man, horse, dog, sheep, cat, cow, pig, hen, woodpecker, shy-poke, frog, turtle, rabbit, crawfish, dove, guinea-pig, rat, fish and embryos of man and some other animals. The material has been ample, but it would have been desirable to examine the peritoneum of monkeys and other animals only obtainable by living in proximity to a menagerie, where one could examine systematically the various genera and species and note the differences. However, material has been sufficient to induce me to believe that the peritoneum of vertebrates is constructed so much alike that it is equally well to select two animals, as the rabbit and frog (cheap and conveniently obtainable), and carefully interpret the phenomena of structures and function of their peritoneum. The endothelia of the fish are like those of mammals. They possess the typical stomata. I examined the peritoneum chiefly over the bladder (of the carp) and it appears very similar to the diaphragm of rabbit. It has vast beds of lymphatics under it. The crawfish has relatively small sized endothelia and they are very compact. The interendothelial line is not as readily dissolved as in other animals. But interendothelial lines in the vast bed of lymphatics lying under the endothelia are easily dissolved in the typical interendothelial space. The fine anatomy and experimental physiology teaches that the peritoneum is a great absorbent organ, a lymph sac. It is a regulator of nutritive fluids and facilitates mechanical motion. It has a constructed apparatus to perform definite functions. The experimental physiology teaches that we should not flush the peritoneum, for it will rapidly absorb the distributed infected fluids.

The dissolving of the interendothelial line of low power into the

double parallel lines with transverse process of high power is very important in the interpretation of the fine histology and physiology of the peritoneum. I have compared the interendothelial space to a railway. The two parallel lines represent the steel rails, while the numerous transverse anastomotic protoplasmic processes represent the ties. The two parallel lines are located along the edges or margins of the cover-plate and is precipitated fluid, albuminous substance bathing the edges of the cover-plate.

The transverse processes cross the interendothelial space or protoplasmic processes binding the cells together into colonies. They are situated below the surface level of the cover-plate and really belong to the protoplasmic portion of the cell which is entirely below the cover-plate. They color dark brown on the surface by application of  $\text{Ag. NO}_3$  from being bathed by an albuminous fluid. That the cover-plate, i. e., the free surface, of the endothelial cell should be hardened, indurated metamorphized portion of protoplasm is analogous to other structures. Its surface in the lymph or blood vessels is frictionized by the lymph and blood stream, while on the free peritoneal surface smoothed by friction and pressure.



FIG 23—(Author.) Horse's omentum. (Oc. 4, ob. 3.) Old, showing many stomata vera and especially two groups around stomata vera 1 and 2. 3 and 4, stomata vera filled with a reddish brown granular matter. Note the numerous stomata vera on left and only two groups on right. 5, stomata spuria. This is carefully drawn to show the numerous stomata vera in one place and few in others. It is in a region where on one side innumerable stomata vera exist; on the other large tracts of germinal endothelium, i. e., growing. It shows that the peritoneum is rapidly changing structure, dying and renewing itself to retain its function. 6, 7, 8, show non-browned endothelia dividing the stomata vera region from the two groups of endothelia.

When we come to the physiologic action of the peritoneum it will easily be noted that all the details of structure will be required to furnish reasonable interpretations of peritoneal absorption and secretion. Especially will we be surprised to know that the peritoneum of the dead animal will absorb, for many hours after death, exactly similar to that of the living. It will be a blow to our views of vital processes, for the histology of the peritoneum must be based on reasonable physiologic

interpretations, i. e., methods of peritoneal absorption and secretion. In the physiologic part of the work will be discussed :

1. The reason of the selection of the diaphragm for the chief path of absorption.
2. Osmosis.
3. Stomata.
4. Imbibition.
5. Infiltration.
6. Endothelial cell vitality, vital forces.
7. The stream of peritoneal fluid toward the diaphragm.
8. Whether the absorptive path of peritoneal fluid is by way of the lymphatics or blood vessels.
9. The structure and function of endothelia of all kinds—lymph vascular, blood vascular and peritoneal endothelia.
10. The relations of the lymphatics to the peritoneum.

## CHAPTER III.

### THE ENDOTHELIA OF THE FREE PERITONEAL SURFACE.

"To the solid ground of Nature trusts the mind which builds for aye."—*Wordsworth*.

The peritoneal endothelia were discovered by Valentin about 1837, and in 1862 Von Recklinghausen announced that a solution of silver nitrate developed dark lines between the endothelia. The term endothelium was introduced by His in 1865 in contradistinction to epithelium. He considered it of mesoblastic origin. The endothelia are arranged edge to edge so as to produce a nucleated membrane, as the peritoneal serosa and the vascular endothelia lining all vessels. Many capillary vessels have only a single layer of endothelia for a wall. Since His introduced the term endothelium instead of epithelium much investigation has been carried on. These investigations, especially those of Kolossow and Paladino, have brought out the idea that the endothelium of the peritoneum is similar to the epithelium as it is covered with ciliated processes, e. g., like the epithelium of the Fallopian tubes or the trachea.

Paladino also announces that the endothelia are covered with cilia. Embryologists are not fully agreed in regard to the strict differentiation between endothelia and epithelia. Kolossow claims that the plates lining the blood and lymph vessels alone should be called endothelium and still later discards the term endothelium entirely. In this work the term endothelium will be used to describe the peritoneal membrane in contradistinction to the term epithelium as applied to animal surfaces which are exposed to the atmosphere or are of epiblastic or hypoblastic origin. The term endothelium will be considered to embrace the plates which line the free surface of the peritoneum, the lymph vascular system, and the blood vascular system. It is of mesoblastic origin and is derived from the middle germ layer of Pander and Baer. Further investigation may alter the associated meaning of endothelium. Endothelium is not a transitional stage between connective tissue cells and epithelial cells, but a permanent condition of connective tissue cells of mesoblastic origin. By careful study with the oil immersion lens, Ag. NO<sub>3</sub>, osmic acid and tannin, it will be found quite suggestive that the endothelium plate and the epi-



thelial cell are very closely related, though the endothelium be of mesoblastic origin while the epithelium is of epiblastic origin. Some investigators (His) hold endothelium for non-genuine epithelium. Kolossow claims that the endothelium is really epithelium, for it approaches it when we can find on the endothelia plates of the peritoneum genuine ciliated process exactly the same as is found on epithelia, as the ciliated epithelia of the Fallopian tubes or the trachea. Yet certainly the cilia of peritoneal endothelia are not so definite to all observers as they appear to be to Kolossow.

Since such investigators as Kolossow and Paladino have found ciliated processes in the endothelial plates of the free surface of the peritoneum, they apply to it the term epithelium. Also since these same observers cannot find the ciliated processes on the flat plates lining the blood or lymph vessels, they apply to them the term endothelia. Hence, with them the peritoneal free surface is lined by epithelium (ciliated) while all vascular tubes are lined by plates known as endothelia. But the above views of Kolossow in regard to the endothelium, I am so far not fully prepared to accept. For example, if we admit that the peritoneal plates on the free surface of the peritoneum are non-genuine endothelium, i. e., it is a transitional form or is an epithelium, it must change our views in regard to the peritoneal cavity being a lymph cavity or cleft, or being derived from a lymph space unless it can be shown that the endothelium lining the peritoneal cavity has changed by evolutionary processes sufficiently to acquire ciliated processes, a stage preceding hairs. So far we have considered the peritoneal cavity a lymph space or cleft originating by fluid pressure and independent action of viscera and body wall, and hence it is lined by plates having, or which should have, the same elemental structure as those which line lymph channels and blood channels. If it be admitted that the peritoneum is lined by epithelium, then the view of the origin of the peritoneal cavity must be entirely changed.

If the peritoneal cavity be not a lymph space, then must the view regarding the pressure of leucocytes also be changed. It would appear that the majority of recent investigators hold that the lining of the peritoneal cavity is of an epithelial character. But such observers as His, Klein, Burdon-Sanderson, Deckhuyzen, Ranvier and many others consider that the plates lining the free surface of the peritoneal cavity should be called endothelia. But I cannot confirm the views of Kolossow, that ciliated processes always exist on the endothelia plates of the peritoneum.

To those who have long studied the plates lining the peritoneal cavity with a microscope and the reagents, osmic acid, Ag. NO<sub>3</sub> and tannin, the statement of Beneden that the peritoneal endothelia and epithelia

are not sharply differentiated will appear significant. To this, as to several other unsettled questions in regard to the peritoneum, there are two sides. It is by no means definitely settled.

In 1865, in the excellent little monograph of His, now before me, it is stated, so far as I am able to find, for the first time, that the flat plates lining the peritoneal cavity, the blood vessels and lymph vessels



FIG. 24—(Author.) Human omentum majus where it was not fenestrated (Oc. 4, ob. 3, Reichert). 1, 1, 1, two nuclei in each endothelium. 2, endothelium; 3, near three intraendothelia stomata; 4, is a granular, germinating cell—a stomaverum. Around it are grouped nine endothelia. Note the nuclei eccentrically located. Observe that this is not a very high power, but the endothelia are enormous in size and very variable. Observe the size of the endothelium No. 5.

are of the same character and, as His asserts in the interest of physiologic knowledge, should be called non-genuine epithelia or endothelia. But some observers, among whom are Kolossow, state that the vascular endothelia (blood and lymph vessels) are absolutely naked, i. e., no cilia exist on them, while all classes of mammals examined by him contained cilia on the peritoneal endothelia. But later Kolossow denomi-

brates the peritoneal plates lining its cavity, the plates lining the blood and lymph vessels, epithelia, and discards entirely the word endothelia. Kolossow claims that he has observed the cilia on the endothelia in motion. Muscatello stated in 1895 that he had never been able to confirm Kolossow's observations. Paladino wrote in 1883 that he had observed ciliated endothelia in the peritoneum. Klein asserts that he observed ciliated peritoneal endothelia in the pregnant frog in the late winter months. I have studied the subject of cilia on the endothelia of the peritoneum of animals with the highest power, oil immersions and the reagent osmic acid, Ag. NO<sub>3</sub>, etc., but I have not been able to demonstrate the long ciliated processes on the endothelia which Kolossow and Paladino claim. I have frequently found irregularly distributed ciliated endothelia in the turtle and frog, especially during the period of gestation. It is true under high power and in well or fortunately prepared specimens we may observe pointed elevations which take on the silver stain strongly. But so far the distinct ciliated processes are not very prominent on the prepared specimens.

Waldeyer, in his "Eierstock und Ei," considers that the peritoneal plates covering the ovary (germinal epithelium) is of an epithelial character and that the whole peritoneal cavity was once lined with epithelial cells or plates of the same character, but by some changes the portion of the peritoneum not covering the ovary became endothelial in character. When one comes to analyze the difference between endothelium and epithelium the difficulty of differentiation becomes at once apparent.

An endothelial plate is composed of a cover-plate, a lamella superficialis, and a subprotoplasmic portion containing the nucleus. The cover-plate is indurated, is metamorphized protoplasm, so modified as to become hard, elastic, hornlike and glistening. It is smooth, but has a granular appearance under high power. The cover-plate is not bound to its neighbor by organized matter, it has no organized anastomosis with its adjacent fellows. The lower portion of the cell, lamina inferior, is composed of protoplasm containing the nucleus and being more granular adjacent to the nucleus. The under portion of the endothelial cell anastomoses with its adjacent fellows by organized processes, by definite portions of jutting-out protoplasm binding the cells into colonies. Now, if as Kolossow and Paladino assert, there be added to this ciliated processes, there are no distinguishing traits to differentiate this endothelial cell from an epithelial cell. If it can be shown that the peritoneal plates and the plates which line blood and lymph vessels cannot be morphologically differentiated from the genuine epithelia of the skin or digestive tract, then there will be no need to name the lining plates of



the peritoneum endothelia, nor will there be any gain in the matter of a better nomenclature.

It may be claimed that the endothelia are analogous to genuine epithelia, because (a) the epithelia and endothelia have both a scaly hard-

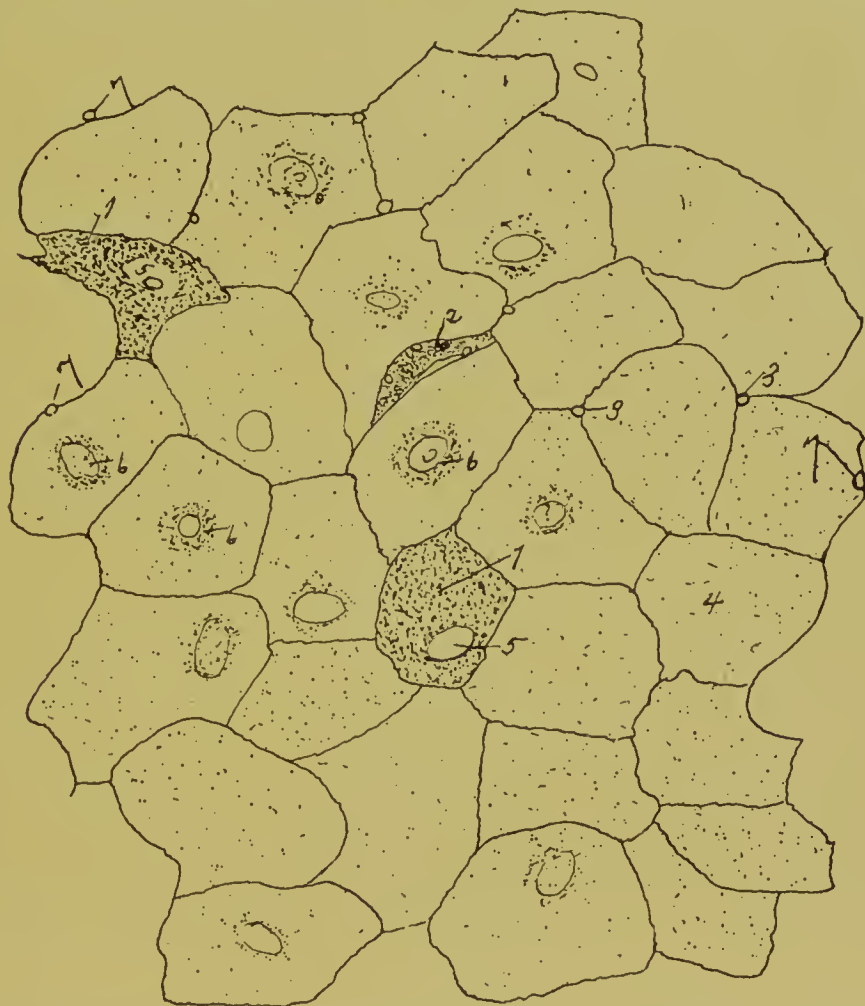


FIG. 25—(Author.) Sketched from rabbit's visceral peritoneum on the ventral surface of the small intestines. Ag.  $\text{NO}_3$   $\frac{1}{2}$  per cent and preserved in formaline. This cut is carefully drawn from Nature. 1, 1, stomata vera; dark granular cells with a nucleus. 5, 5. Note that Nos. 1 and 1 have grouped around them five endothelia. The dark, granular cell 1, may be (a) growing, or expanding over the surface of the adjacent endothelia or (b) shimmering through the transparent endothelia (c) the granular cell, 1, may be simply filling up the aperture, 1, which lies at the common junction of the 5 endothelia. 3, stomata vera which does not show any polyhedral granular protoplasmic cells. They may have fallen out or the protoplasm may have actively retracted. 6, 6, 6, nuclei, one with a nucleolus. 7, 7, 7, stomata spuria simply lying on an inter-endothelial line. So far as I have examined the endothelia on the ventral surface of the intestine it is very irregular in shape and size. No. 1 is likely a point where endothelia are regenerated to supply the place of degenerating comrades.

ening on the surface, metamorphized protoplasm; in other words, as Kolossoff terms it, a cover-plate (Deck-plate), a lamina superficialis; (b) both endothelia and epithelia have an organized connection, each cell is anastomosed to its fellow by protoplasmic processes which can



be well demonstrated in endothelia by appropriate reagents and the oil-immersion lens (best in the frog). (c) It is claimed by Kolossow and Paladino that the endothelia possess cilia. Hence, endothelia and epithelia are analogous and both should be termed epithelia. The many layered epithelia are only samples of cover-plates before they are shed or cast off.

Waldeyer demonstrated that the covering of the ova are epithelia as did Pflueger, and that the whole peritoneum was originally lined with epithelia, which disappeared and was replaced by endothelia from the subjacent connective tissue. Now, if the cells covering the ova be epithelia, it is argued by analogy that the remaining portion of the flat cells which line the peritoneum is epithelium. However, though the endothelia and epithelia have many things in common and are even difficult to differentiate, it is more convenient to retain the names endothelia and epithelia for the substantial reason of their origin. Endothelia comes from the middle germ layer. Epithelia comes from the two lateral germ layers. It is less confusing to name cells from their place of origin. An illustration may be drawn from the elements of matter. Carbon may exist in two different forms, as charcoal and as diamond, and it tends to clearness of expression to retain both terms, charcoal and diamond, instead of attempting to describe both substances by the one word, carbon.

Should the discoveries of Kolossow, Paladino, Leydig and others, as to the cilia being found on the endothelia, be confirmed and be found generally in the peritoneal cavity, it does not seem to add sufficiently to anatomy or physiology to rechristen all cells epithelia. For no one has, so far as I know, even suggested the physiologic use of ciliated endothelia in the peritoneum of mammals, for cilia can hinder as well as hasten currents. Besides, some go so far as to designate naked vascular endothelia as epithelia with apparently non-sufficient reason. Hence, the endothelia are not designated endothelia because they are ciliated. Moreover, cilia are doubtless, mere elongated protoplasmic processes, a part of the cell itself, and perhaps little significance should be attached to them as an essential attribute.

In dealing with the peritoneum or serous cavity, we are engaged in studying a structure derived from the middle germ layer or mesoblast. It is a closed space with shining, smooth walls and contains a certain amount of serous fluid. The characteristic of this serous membrane, peritoneum, is that its walls are lined by flattened cell plates supported on a thin layer of cellular tissue. The smooth peritoneal wall may be closely and intimately connected with the subjacent structure at one point but very loosely connected at another. In regard to the independence of the peritoneal membrane an old strife has long existed. Bor-

deau claimed that the existence of the peritoneum was due to mechanical pressure and friction arising from growing viscera. But Bichat combatted the idea and showed that the peritoneum owed its structure and physiology to the original process of development. His was one of the first, if not the first, to declare the peritoneum to be lined by flattened connective tissue cells, merely modified connective tissue cells. The peritoneal covering allows the viscera to project into its space, but all viscera projecting in its cavity are entirely covered or faced by the membrane; that is, no organ lies within the peritoneal cavity.

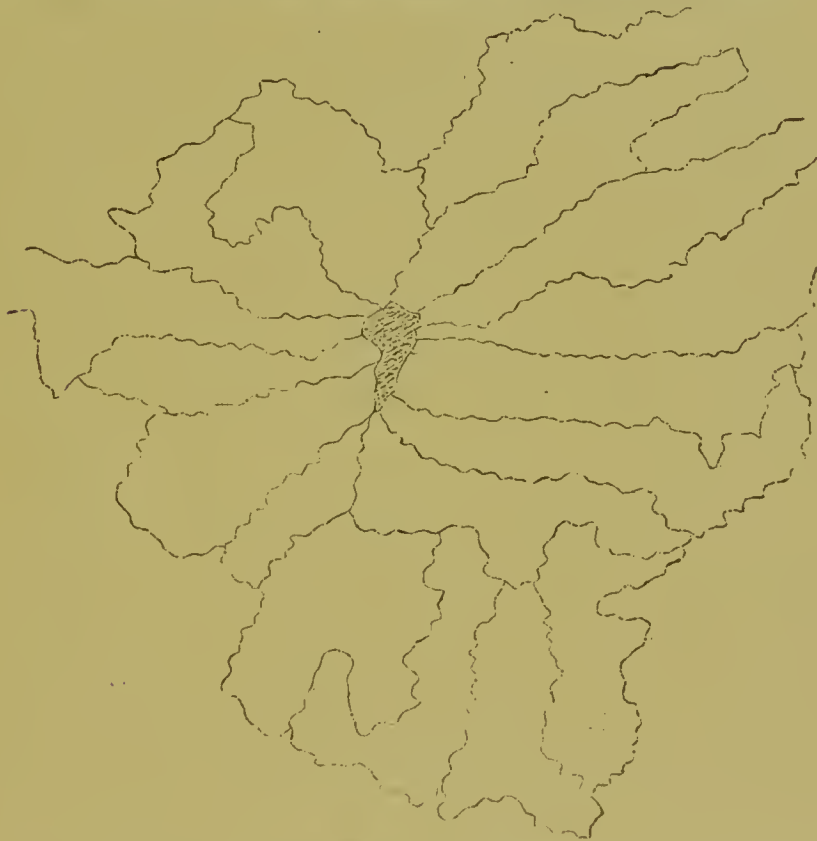


FIG. 26—(After Peter Nikolsky, 1880.) Is drawn from a male frog's abdominal parietal peritoneum. It represents a stoma verum around which 11 endothelial cells are arranged. The stoma verum is composed apparently of two protoplasmic granular cells.

The peritoneal membrane lines a lymphatic space, or a cleft, which exists in various numbers in the middle germ layer and mesoblast. These lymph cavities and lymph spaces must be strictly mesoblastic in origin. If it can be shown that the epithelia and endothelia have a common structure and origin, it will be a real advance in medical science.

His distinctly declares that all the cell layers which line closed spaces, lymph or vascular spaces, as the peritoneum, have much in common and they differ from the first from the cells of the epiblast or hypoblast so much that they should be called endothelia. The place

of origin of cells should be considered in naming them. The long famed embryologist, His, states that the cells lining closed cavities (peritoneum) differ so much in their embryological origin from epithelia that they should have a distinct nomenclature. Both epithelia and endothelia arise out of lymphoid cells. The lymphoid cells quickly take on their flattened shape, endothelial plates, and their transparency. When the endothelial plates have reached their final object, they experience very little further change. The epithelia act differently. Their layers increase and may be many and thick, and the dark nucleated contents differ from endothelia. Besides, the shape of epithelia assume cylindrical, prismatic or various other shapes and grow independently of the surface, generally perpendicularly to it. Again, the function and products of the endothelia and epithelia differ. Epithelia produce various kinds of secretion. Endothelia produces no secretions, or at least very different from epithelial secretion. There is a vast difference in function.

The epithelial cell is a highly active secretory structure and also with its cilia performs the office of making fluid currents, as in the Fallopian tubes. Pflueger tried once to show that the peritoneum was a secretory gland, for what reason it is difficult to explain. The endothelial layers are so ordered that serous fluids pass through their walls in either direction depending on osmosis, structures in their wall, and pressure. This cannot be said of epithelial layers. It is characteristic of the endothelial layer that it allows albuminous transudate. It is characteristic that normal epithelial cells do not secrete albumen. Diseased epithelial cells secrete albumen, as may be observed in the kidney and mucosa of the digestive tract. Neither epithelia nor endothelia form a continuous, non-interrupted membrane, as the chyle passes through the digestive mucosa to the lacteals, and solids suspended in fluids will pass out of the peritoneum into the lymph vessels. The vulnerability of endothelia is much greater than epithelia. The endothelia is capable of virulent and fatal infection to a greater and more rapid extent than epithelia. Endothelia are less liberally supplied with nerves and vessels than the epithelia. The endothelia are capable of functioning for a period and then becoming obliterated, as in the umbilical vein, the arteriae hypogastricae, processes vaginalis, and ductus Botalli. Such structures, i. e., the endothelia, return to the original connective tissue or as it is generally expressed, to a fibrous cord. In parts which are lined with epithelia and have only a temporary function in the animal economy, the epithelia are more persistent and dangerous to subsequent life, as the urachus, appendix, the Wolffian remains, the pronephros, the parovarium, the remnant of the ductus amphalo mesentericus or Meckel's diverticulum. Epithelia in body remnants

are likely to partially persist and degenerate into pathologic conditions. A serous cavity, the peritoneum, is a vascular cavity, and both serous and vascular cavities are lined with endothelia. Lymph cavities and vascular cavities are appendages of each other. The serous cavity is a supplement to the vascular cavity and both have the same kinds of lining plates—endothelia. As blood cavities (vessels) are characteristics of the mesoblast, so are lymph cavities characteristic of the mesoblast, and the lining membrane of both cavities should be denominated the same—endothelia. It is characteristic for the mesoblast to form closed spaces which contain lymph. Such cavities are vascular spaces, and as the lymphatic system is a secondary appendage of the blood system, it originates from the mesoblast as the blood vascular system does



FIG. 27—(After Oedmansson, of Stockholm, Norway, 1863.) Epithelium (endothelium) of the lymph sac of a frog with some openings and colored points between the cells. Silvered. One point lies at the common junction of several endothelial cells and consists of a black dot and a semi-circular or crescentic ring about it. All other dots are situated in the intraendothelial space.

and both are consequently lined by a similar membrane—an endothelial membrane.

The amphibians have large lymph spaces in addition to the peritoneum, almost vieing with it, and all are lined with endothelia just as the blood vascular system. Hence, viewing the subject from an anatomic, physiologic and embryologic standpoint, it appears to me that we should retain for the flat plates which line the peritoneum, lymph vascular system and blood vascular system the name endothelia. In other words, all that arises from the mesoblast should be termed endothelium, and all arising from the hypoblast or epiblast should be termed epithelium. To consider the plates lining the closed lymph cavities (interstitial spaces) of the mesoblast, epithelia, is not in accord with the present stage of embryology, and it must first be proven that the closed



mesoblastic cavities are not lymphatic. Also a new explanation will be demanded for the relation of the peritoneum by organized channels with the subjacent lymph channels.

But leaving out of view the disputed question of preformed, organized openings between the peritoneum and the subjacent lymph channels, all the reasoning up to date does not explain away the fact that solid, insoluble particles, suspended in fluid and injected into the peritoneal cavity, are transported in a few minutes to the subjacent diaphragmatic lymph channels. To say the least, the communication between the peritoneum and lymph channels must be free. It is suspiciously true that the digestive epithelia are very intimately free in their communication with the lacteals (intestinal lymphatics). The methods of transportation of the chyme through the epithelia into the lacteals has been in the field of medical polemics from the days that the ingenious Lieberkuhn announced open lacteal mouths, until today. It may be stated that the structures of the endothelia are much more complicated than was previously supposed, and more perfect lenses as well as untried reagents will doubtless reveal many unknown facts. However, I gained far more success by persistently following a few definite lines of investigation in regard to the endothelia than by trying many different methods. Amphibians (frog or turtle) and one mammal (rabbit) should be continuously employed, yet the turtle's peritoneum is the most typical of all animals to acquire knowledge of the lymphatics or varied interstitial spaces. The microscope from low power to 1-15 oil immersion with  $\text{Ag NO}_3$ , osmic acid and tannic acid with glycerine, carefully employed, reveals wonderful structures in the endothelia. Added to this, the distension of the organ by force, as air, which is covered by the endothelia, and one can by subsequent examination discover organic, anastomotic connection of the inferior portion of each endothelial cell with the adjacent ones. Stretching the membrane presents similar results. The endothelial plate can be observed to be composed of two distinct parts, the lamina superficialis or cover-plate and the lamina inferior or protoplasmic portion containing the nucleus. The superficial portion of the endothelial plate becoming hardened, or indurated, gives the endothelia a suspicious resemblance to epithelia, especially of the skin, which sheds its cover-plates with the wear and tear of time. It is quite probable that the endothelial cover-plate is normally shed with the old age of the plate and renewed. I know the cover-plate of the endothelia are rapidly shed in peritonitis, and rapidly renewed, for it is easy to find organized bands of peritoneal exudates completely covered with a new mantle of endothelial plates. The new endothelial plates appear to be of less size than the original ones, at

least in the specimens which I examined. This is perhaps in the younger stage resembling germinal endothelia.

Perhaps no distinction of function or structure would arise by discussing the organic connection which exists between epithelium and endothelium, as their connections are similar. Kolossow declares with surprise that until now the cilia of the peritoneal endothelia were overlooked. Yet it is not surprising to me when but one other observer, Paladino, discovered ciliated endothelia in local portions of the peritoneum only. The best of observers so far do not confirm all of Kolossow's assertions of universal ciliated peritoneal endothelia. In pregnant frogs and turtles I have found in localized peritoneal endothelia bunches of cilia which do not cover the whole of a single endothelial plate. The cilia resemble hairs on a wart, i.e., are localized both on particular endothelia and on distinct portions of one endothelial plate. They seem to arise in small bunches or tufts on a localized portion of the plate quite adjacent to the large frictionizing fruit or gestation sac and especially on the mesenterium of the ova sac. In the endothelia of any

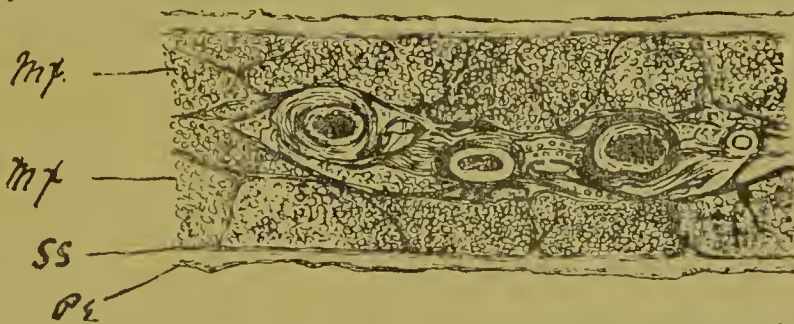


FIG. 28—(After Toldt.) A transverse section through the mesentery of the small intestine of a boy 4 years old. Mp and Mp, membrana popia mesenterii. SS, subserosa. Pe, peritoneal layer.

mammal as one observes the surface of the plate through the microscope, it is easy to observe dark brown dots here and there which no doubt are identical with Kolossow's "Kurtzen Harchen" or short cilia. It seems to me that Kolossow exaggerates the phenomenon of cilia on the endothelia when he asserts that the cilia of the endothelia endow them with sufficient analogy of structure to term them epithelia, and that they exist universally in the peritoneum. Especially suspicion is called to the subject of universal ciliated peritoneal endothelia when the author of this view himself states that he cannot find the ciliated endothelia in birds, reptiles nor fishes, neither does he find them in amphibians except in the axolotl. One would expect that the lower animals would be possessed of the typical ciliated endothelia, but here it is the reverse. The ciliated endothelia would naturally be looked on in the highest mammal, man, as a remnant. If ciliated endothelia were an acquisition by evolution as needed they would have been acquired long before man was reached in the ascending complicated scale of animal life.

Again, it is announced that in many of the embryos examined the ciliated endothelia are not present. Hence there appears a discrepancy in the significance and constancy of the ciliated peritoneal endothelia. To be present in higher vertebrates and not in the lower, to be absent in the embryos and present in adults does not accord with our present views of embryology or evolutionary processes.

By careful examination in fresh specimens with fresh serum Kolossoff himself could not detect movements of these cilia, and I find no record of any other investigator either finding the universal ciliated endothelia in the peritoneum or detecting their motion. We suggest that further investigation may disclose facts of importance.

There exists much variation of opinion in regard to the origin and formation of the mesoderm. Some embryologists claim that the mesoderm arises from the entoderm and others from the ectoderm. But it is fairly agreed of late that the mesoderm arises independently from the primitive streak. Now the mesoderm contains two distinct cavities, which are lined by cells which are designated as mesothelium. From this mesothelium is thrown out that which composes the mesenchyma or connective tissue of the embryonic types. We have then to consider the two subjects of mesoderm, the mesothelium and the mesenchyma. Now if we are going to change the term endothelium, would it not be as well to designate it mesothelium? Where the coelum or body cavity found in the mesoderm arises, the mesodermic nucleated cells form a layer, the mesothelia, lining the coelum cavity; the cells assume a mere epithelial arrangement, but do not assume the role or function of genuine epithelium. The complex series of cavities in the mesoderm soon coalesce and form the whole peritoneal cavity. The whole coelomic cavity is now lined by the cells of the mesoderm which form a kind of sheet known as the mesothelium. Step by step the mesodermic cavities formed by fluid pressure and coalescence of septa, gradually enlarging the body cavity or peritoneal cavity, become lined by mesothelial cells which finally become the endothelial. The excellent but little known monograph of His in 1865, "*Die Haute und Hohlen des Körpers*" ("The Membranes and Cavities of the Body"), was rich in new material. His divided the mesoderm into archiblast and parablast. Approximately the archiblast of His means the mesothelium of today, and the parablast of His means the mesenchyma. To me this very small essay has been rich in suggestions. Accumulated observations have modified the views of His to some extent. Minot, in his excellent embryology (1892), asserts that the peritoneum is derived from the mesoderm.

#### THE ENDOTHELIAL CELL.

Valentin discovered the endothelial cell of the free peritoneal sur-





FIG. 29—(A. Kolossow, 1895.) Endothelial cells from the anterior abdominal peritoneum of a perch. Showing anastomotic processes in the interendothelial spaces.

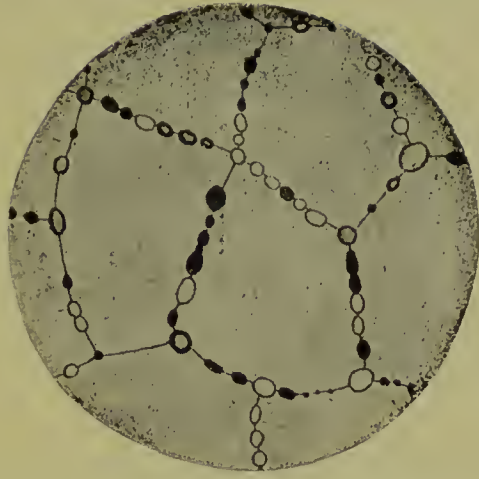


FIG. 30—(A. Kolossow, 1895.) Endothelial covering of the small intestine of a pole-cat, silvered while in a state of distension. The silvered interendothelial lines are interrupted by stomata spuria (stigmata) appearing in the form of rings and black points.

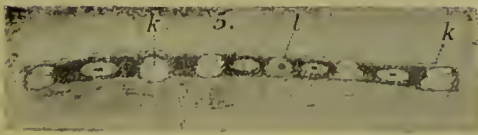


FIG. 31—(A. Kolossow, 1895) is a vertical section from the peritoneum on the turtle's stomach. K, K, represent intercellular canals on the cross section. I, a transversely cut leucocytic process. The superior fine line is the cover-plates. The tissue below the intercellular canals is the subserous tissue.

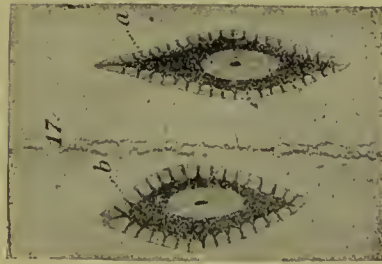


FIG. 32 (A. Kolossow, 1895.) Isolated endothelial cells from the artery (a) and from the vein (b) of a rabbit, showing the anastomotic protoplasmic processes projecting from the edges of the cells.





face. It consists of two distinct layers, lamina superficialis et inferioris, parts held together by protoplasmic processes. (a) The upper part consists of the endothelial plate of a hyaline-like membrane of an elastic nature. The plate is not homogeneous, but contains an intercellular network of irregular fibrils visible under high power only. The upper portion of the cell we may call the lamina superficialis or cover-plate. It can be easily brushed from its bed by using a fine wet brush or a little cotton on a toothpick. Even slight inflammations, peritonitis, quickly loosens it from its bed, leaving behind a depression in the subjacent protoplasm. The cover-plate does not contain the nucleus of the endothelial cell. It appears more thick and distinct at its edge than at its center. Stained with  $\text{Ag. NO}_3$  it presents a brown or occasionally a dark color. If the plate be young, it turns a dark brown color with silver salt. The plate can be noted to be



FIG. 33—(Handbook for the Phys. Lab. Vol. II., 1873.) A silver preparation from the omentum of an ape, showing groups of germinating endothelial cells amongst the ordinary large endothelial elements which cover the trabeculae (b). (Oc. 3, obj. 5. Tube half drawn out.) This specimen shows elegantly the mosaic-like groups of germinating endothelia, such as is found in its typical condition on frog's mesentery. a, smaller trabeculae. In 1866 Schweigger-Seidel called such groups of germinating cells ciliated cells (Flimmerzellen).

very elastic under the microscope. It springs backward and forward under the pressure of water currents. It is thin and transparent, having in general a polygonal shape. The cover-plate is the hardened indurated smooth portion of the endothelial cell. Its smooth, flat surface is due to wondrous delicate movement of the viscera. It is metamorphized protoplasm. Viewed under the microscope after being fixed with osmic acid and tannin and stained with silver nitrate, it presents a brownish aspect dotted here and there with dark spots which are supposed by some to be cilia, or protoplasmic projections of the cell. The cover-plate does not anastomose with the adjacent plates by protoplasmic processes, but its edges simply lie in contact with its fellows. The anastomotic connection belongs to the protoplasm subjacent to the plate. From the circumstance that the cover-plates do not anastomose

with their adjacent fellows arises the fact that there is a clear space of separation between them which can be easily seen in prepared specimens, especially of the frog. By changing the microscopical focus one can see the dark transverse anastomotic lines connecting the subjacent protoplasmic portions of fellow cells. When the cover-plate is torn from its bed by a brush and subsequently its bed is stained with  $\text{Ag. NO}_3$ , one can observe the broken protoplasmic threads which held the cover-plate to the protoplasmic portion of the cells stained quite dark with the silver. These threads on the projections of the subjacent protoplasm thrust through the cover-plate and doubtless constitute what is known as cilia. The quite dark dots show the protoplasmic processes to be well loaded with albumen or new protoplasm, and hence take the stain of  $\text{Ag. NO}_3$  on readily. It is perhaps not quite correct to say that the edge of one cover-plate does not anastomose with its fellow by protoplasmic processes, but that the anastomosis is exceedingly fine and small and certainly belongs chiefly with the under edge of the cover-plate. The anastomosis of one cell to another by protoplasmic processes begins by very fine lines of protoplasm extending from the under surface of one cover-plate to its adjacent fellow, and the size of the anastomotic processes increases as they descend from the surface. The anastomotic protoplasmic processes, stained black by  $\text{Ag. NO}_3$ , are quite equidistant from each other in some specimens while in others they are very irregularly distributed. I have drawn these processes in but a few figures, as other purposes were in view.

In regard to the upper or indurated portion of the endothelial plate being separated from its fellow, it appears that there are two men who did special labor on the subject. Ranvier worked out and published the idea that the upper portions of the endothelial plates were not in contact, not held together by anastomosing processes except perhaps the most inferior parts of the plate. The cover-plate is held fast to the inferior protoplasmic portion by anastomotic processes of the protoplasm containing them, the protoplasmic portion of the cell being strongly connected by radiating protoplasmic processes. It appears that Dr. A. Kolossow of Moscow, Russia, had worked out independently exactly the same views as Ranvier, but so far as I can observe Kolossow went a little further than Ranvier. Ranvier, however, notes that in inflammation the cover-plate first falls off and the underlying protoplasm swells up with its radiating anastomotic processes, and the whole endothelial cell (except the cover-plate) appears prominently as a stellate cell. Ranvier claims that after the inflammation the cells fall off and the few remaining ones reproduce, regenerated by karyokinesis. However, he notes that in a few days the adjacent connective tissue cells become transformed into endothelial cells.

(b). The under portion of the endothelial cell, the lamina inferior or protoplasmic portion, contains the nucleus. It is the real, living portion of the cell containing the reproducing and assimilating portions of the cell, the nucleus. The protoplasmic part of the endothelium rest on the membrana limitans. The protoplasm is more granular adjacent to the nucleus.

The nucleus lies imbedded in the protoplasm of the cell some distance below the cover-plate. It is in general situated in the center or adjacent to it. In particular cases it is situated excentrically, and even at the very edge of the cell or at its end. No reason is forthcoming why the nucleus is so frequently located excentrically in the protoplasm of



FIG. 34—(Author.) Omentum majus of new-born, which died a few minutes after birth and was stained twenty-four hours later with Ag. NO<sub>3</sub>. (Oc. 2, ob. 2a R.). 1, stoma verum with dark clumps as 2, projecting into the stomata which I take to be granular cells. 3, rift between endothelial cells and debris. 4 points to a part of the stomata which appear under the endothelial cells 5 and 6 and is shimmering through. Note the length of cell 7, which extends so wide that the microscope field does not cover it. This stoma verum is filled with granular matter which takes on the stain of Ag. NO<sub>3</sub> vigorously. Stomata spuria 8, 9, 10, 11; 12 shows intraendothelial stomata. 13, 14, nuclei.

the cell. The idea of unequal pressure, perhaps, explains the most. The shape of the nucleus is mostly oval, but may be round or club-shaped. It may be constricted at some portion, chiefly in the middle, and it is not infrequent to find two nuclei in each cell. This, no doubt, shows a state of reproduction. The nucleus is a vesicle. It is contained within a limiting membrane. It contains a network of fibrils which is called an intranuclear network, as the network within the protoplasm of the cell is called an intercellular network. The nucleus frequently contains one, two, three or even more nuclei. The bright, shiny spots in the nucleus may be optical sections of fibrils existing in the intranuclear network. But no doubt these clear spaces may be perforations in the membrana limitans on which the endothelia rest. The nucleus is characteristic for its smoothness and even contour. The constriction of



a nucleus is an indication of division or reproduction. The nucleus is surrounded by a distinct nuclear membrane and contains a semi-fluid substance in which are suspended the nucleus and an elaborate network of fibrils. The size of the nucleus is very variable in the endothelium. Sometimes it occupies a third of the size of the plate. In well formed specimens, with osmic acid, Ag. NO<sub>3</sub> and tannin and an oil immersion lens one observes a beautifully distinct nucleus and the protoplasmic portion of the cell sending out its radiating processes to connect other cells to it. The protoplasm or body of the cell may be represented by the hub of a wheel, while the radiating projecting, anastomotic protoplasmic processes may be represented by the spokes. The cover-plate with the protoplasm containing the nucleus forms one individual unit, a whole endothelial cell.

The peritoneum, composed of endothelium in general, is a nucleated membrane, but other membranes are composed of endothelial plates as blood vessels, lymphatic vessels and the lining of lymph sinuses. Many capillaries have their wall composed of simply a single layer of endothelium, i. e., flat, membranous nucleated plates joined edge to edge so as to compose a hollow tube for fluid conduction. The peritoneal endothelium is derived from the middle germ layer of Pander and Baer. In this chapter will be discussed that form of endothelium which lines the free surface of the peritoneum. In other words, we will consider the connective tissue cell, which is flat and smooth on one side, as the peritoneal endothelia are only modified connective tissue cells. The origin of the peritoneal cavity is no doubt due to fluid pressure in lymph spaces and visceral motion independent of the body wall. I mean by this that the fluid pressure in lymph spaces became so constant and so vigorous that the fine partitions gradually atrophied or became absorbed until the space coalesced so much that a lymph space (the peritoneum) of more or less size arose. This enlarged space is lined by what we are terming endothelial plates. Added to this, the viscera adjacent to these developing and coalescing lymph spaces became more and more independent in their movements relative to the body wall. Thus by increasing development of lymph spaces and by increasing independent motion of viscera and body wall the great lymph sac, the peritoneal cavity, was formed with its smooth lining endothelial plates. The evolutionary process in producing a monster lymph sac with smooth walls was an act of adaptation of the viscera to their environments. The needs of progressive visceral growth and fixation of body wall was motion, and this was accomplished by fluid in a smooth sac. It reduced friction to a minimum and increased motion to a maximum.

The endothelium and its nucleus are so transparent and homogeneous that they can be clearly observed only with reagents. One can

observe the outlines of the protoplasm and nucleus in fresh membrane. But it is practically a waste of time to study the endothelium of the peritoneum without reagents. We shall consider the endothelium as a modified connective tissue cell endowed with a fibrous and elastic nature. The endothelium is simply a connective tissue cell flattened out and indurated on one of its surfaces. From long adaptation to environments of pressure and friction the peritoneal endothelia have acquired a smooth surface. The cover-plate with its underlying protoplasm, holding a nucleus within its embrace, constitutes a single unit, an endothelial cell, the other chief point which attracts our interest in the construction of the peritoneum.

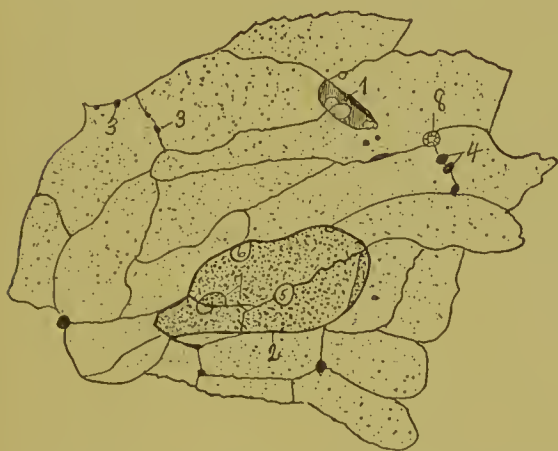


FIG. 35—(Author.) Drawn from omentum of new-born (Oc. 2, ob. 8a R.). The cut represents two stomata vera, 1 and 2. They appear to me in different stages owing to age. 3 and 4, stomata spuria. 5 and 6 two small cells perhaps constricted off during growth. 7 may be a precipitate, but is not clear. 8, stoma verum. Stoma 2 is in a rapid field of growth. Stoma 1 is filled with polyhedral granular cells. The great irregularity in size and shape of the new-born omental endothelia is here apparent. Characteristic-endothelial grouping is typical in the omentum of the new-born.



FIG. 36—(After Peter Nikolsky, 1880.) Represents endothelia from a male frog's pylorus. (a) are what Nikolsky calls protoplasmic bodies. They are what other authors call stomata vera, or granular, protoplasmic cells lining a canal leading from the peritoneum to the sub-peritoneal lymph channels. Some of the protoplasmic "bodies" may be germinating endothelia.

By peritoneal endothelia is implied a layer of flattened smooth cells lining its free surface. The plates of endothelia are so arranged edge to edge that a continuous, non-interrupted membrane is produced. Peritoneal membrane is different from mucous membrane or the membrane which lines glandular cavities. Mucous membranes possess epithelium and arise from hypoblast or epiblast. The peritoneal membrane, serosa abdominalis, is derived from mesoderm or mesoblast, from mesothelium. The naked eye appearance of normal peritoneal endothelia is of shiny, glassy tissue. It is polished and reflects light. The eye can distinguish no lines or unevenness on its surface, it is smooth. In color it is grayish white, pink or pearly, depending, however, on its vascular

condition to some extent and the subjacent organs. It is transparent, and many structures may be observed under it. The endothelial layer itself does not change so much in appearance as the subjacent structures. Fat makes it appear yellow; pigment cells, dark, while bile-stained subjacent structures give it a variegated color. In short, its color arises from subserous organs and conditions. Previously diseased endothelia returned to normal may even show a mottled or slightly opaque condition.

To the touch the endothelial membrane feels smooth and moist. It is slippery from the viscid condition of its secretions. It is moist and slippery, sufficient to produce the least amount of friction in moving viscera. It is so thin that one could perhaps not distinctly feel less than four or five layers between the fingers and thumb. The outer surface of the peritoneal membrane is rough and attached to viscera or parietes. The best methods to observe the thin portions of the endothelial layers is to allow it, especially the omentum majus and gastrosplenic, to float in a large capsule of water, where the thin, delicate membrane will float about, sometimes almost invisible. On exposure to the atmosphere it quickly becomes dry, wrinkled and brittle. A brownish color appears to rise on becoming dry. The extent of the peritoneal endothelia is not very much less than the extent of the skin. The endothelia, i. e., the connective tissue cells, the endothelial membrane in the foetus of a young child rests on a remarkable loose cellular bed, hence the numerous displacements in early foetal, embryonic and early post-natal life. One can with the finger glide the membrane often 1 to 2 inches in various directions without destroying its integrity or power of returning to normal. Cells flattened and smooth on the free surface are the essential elements of the peritoneum. Without the endothelia the peritoneum is robbed of that element which endows it with physiology and is the chief element which prevents the invasion of disease. The membrane presents almost the same appearance in all mammals.

The preparation of the peritoneal endothelia, and the interpretation of its appearance under the microscope, is the first step to a knowledge of structure and function and it is the second step to make such knowledge of practical benefit to combat peritonitis, the vigilant enemy of mammalian life. The first precaution in preparation for the observation of the peritoneal endothelia is that the membrane be as fresh as possible. The second is that all possible trauma to the membrane be avoided; manipulating, dragging and tearing must not be practiced. The membrane should be handled only in a fluid medium, and even in the fluid with as little movement as is compatible with results. For the fresh specimen the animal may be killed, and the parts of the peritoneum to be examined should be gently cut out with a very sharp scissors and



examined in serum from the abdominal cavity, or placed in a large capsule of distilled water. Small bits of the membrane can be snipped off and allowed to float on the slide and then examined in serum, water, or a drop of glycerine applied to the under surface of the cover glass just before the cover glass is placed over the specimen. A solution of  $\frac{1}{2}$  per cent. common salt (Na.Cl.) solution is an excellent medium. Much knowledge may be gained by the microscopical examinations of such specimens; in fact, all the knowledge of peritoneal structure rested on

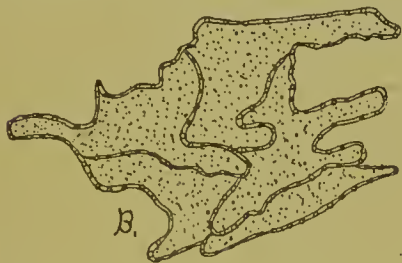


FIG. 37 — (Author.) Is drawn from a toad's stomach to show the variation of shape of the endothelia over an organ of wide contractile and expansile power which goes through its range of expansion and contraction quickly, indicating that endothelia adjust their shape to environments. Drawn under 1-15 oil immersion. Ag.  $\text{NO}_3$  applied. No nuclei could be discerned. The interendothelial space in the toad is exceedingly fine, and large lymph spaces lie immediately under the endothelium at numerous points. Yet all outlines of the endothelia are distinctly marked by the Ag.  $\text{NO}_3$ . The toad's peritoneum is quite thick on account of the large amount of fibrous and muscular tissue running between its mesenteric blades. The amphibia have a large amount of muscular and especially fibrous bands in their peritoneal supports. The turtle is abundantly supplied with vast bands of fibrous tissue in its ligamentum peritonei.



FIG. 38—(Author.) Dog's gastro-splenic omentum. 1,1, stomata verum cells. Germinating endothelia around a stoma with multiplying round, nucleated cells; 2 to 2, nuclei and in some cells two may be seen; 3, stomata spuria, some of the cells project entirely above the endothelial surface and even require a different focus than the flat endothelial cells of the common surface; 4, points to a space where the endothelia has been shed; 5,5, sproutings of new cells; 6, appears to be the common endothelia surface not yet covered by the germinating endothelia.

such examinations up to 1860. But I shall base my investigations and interpretation on peritoneal endothelia treated with Ag.  $\text{NO}_3$  solutions, as Von Recklinghausen taught thirty-five years ago. The Ag.  $\text{NO}_3$  solution should be  $\frac{1}{4}$  to  $\frac{1}{2}$  per cent., always in distilled water and better if made fresh every two weeks. (Two grains of Ag.  $\text{NO}_3$  to an ounce of distilled water makes a good stain.) For example, a rabbit is killed by bleeding or some other method. The abdomen is opened with care and a solution of Ag.  $\text{NO}_3$  is poured over the peritoneum in situ, while the animal lies on the back. No viscera or peritoneum should be touched or traumatized before the Ag.  $\text{NO}_3$  solution is poured on the perito-



neum, and it should remain 2 to 15 minutes. But by experience and results, one soon learns how long to leave the silver solution on and how much sunlight should be allowed to shine on it. The results depend on the strength and time the silver solution remains on the endothelia and the duration and intensity of sunlight. I have examined specimens two months after mounting in glycerine, which were continually exposed to light, and found them improved with age. The silver-stained endothelia should remain in distilled water for an hour before placed in common water, for the salts in common water interfere with the working of the Ag. NO<sub>3</sub> solution. If one wishes to check the effect of the silver solution on the membrane it should be placed in a Na.Cl. solution  $\frac{1}{2}$  per cent. Any trauma is liable to desquamate the endothelia, but it will be liable to disturb the peculiar structures known as stomata vera, as well as the interendothelial space, and as this last named structure is pliable and delicate, it can also become disarranged.

We will now consider the peritoneal interendothelial space. What appears on examination of a silver-stained endothelial specimen through a microscope? First, irregular dark lines appear which separate brownish spaces, the endothelia, from each other. These dark lines are said to be precipitated albuminate of silver, the interendothelial substance being of an albuminous nature also, they represent darkened protoplasmic anastomotic processes. Some have claimed that the dark lines are only precipitated albuminous fluid substance which exists in furrows on the endothelial membrane, for all admit a serous fluid exists on the surface. Schweigger-Seidel enunciated and defended this view persistently, and as proof said if the peritoneal endothelia be first washed with diluted glycerine or diluted sugar solution, the silver solution will not produce the dark lines. However, Klein positively denies the assertion by repeating the rinsing of the membrane and then says the silver solution produces the lines as before. Also, that by rinsing the peritoneal endothelia with water the silver solution will still produce the dark interendothelial lines. I have repeatedly washed the peritoneal membrane with water, but it does not prevent the silver staining the interendothelial lines. Hence, this is sufficient proof that the dark interendothelial lines are not on the surface at all but in the semi-fluid substance or protoplasmic matter between the plates. I can say that I have repeatedly watched the effect of the silver salts on the interendothelial lines from scarcely perceptible dark lines until a week later, when the lines have thickened in breadth and also to some extent shown some connection with the substance on the endothelial plate. The endothelial plate will gradually brown deeper and deeper from the circumference of the plate, i. e., from the interendothelial line toward the nucleus of the cell plate. But the dark, irregular, interendothelial lines

appear first. In many endothelia the nucleus remains an oval or round clear space, i. e., the silver solution does not brown it but the circumference of the nucleus is intensely brown. The only reasonable explanation of this phenomenon is that the protoplasm adjacent to the nucleus is younger or more granular, and that the nucleus is higher than



FIG. 39—(Author.) Drawn from pleural surface of dog's diaphragm (Oc. 4, obj. 8a, Reichert). It is intended to illustrate a lymphatic vessel, 1, 1; a germinal bud 9-7 and stomata vera 5, 5, 5. Note the regularity of the endothelia 2, 2, 2, covering the lymph vessel 1, 1. One of the endothelium has a large nucleus and a nucleolus. But the interesting part of this figure is the beautiful and distinct germinal bud sprouting up at the edge of the lymph vessel. The bud projects entirely above the common surface endothelium 8, 8, 8, 8. The stalk of the germinal bud is made of 3 cells, 6 and 9 and a small cell at its foot. As the stalk lengthens, it expands, spreading itself over and covering up the common surface endothelium. 7, 7, 7, 7 shows the bud endothelium, which is very brown from the Ag. NO<sub>3</sub>. This figure illustrates how the endothelium is regenerated to supply the places of dying comrades. 1, lymph vessel; 4, nuclei; 5, 5, 5, 5, stomata vera; 6, pedicle of bud; 7, 7, 7, endothelia of bud; 8, 8, 8, 8, endothelia on which the bud rests. The bud or germinating endothelia require different focus. The bud is very granular and quite distinguishable from adjacent endothelia, or origin of stalk. Dog 3 months. (Oc. 4, ob. 8a, R.)

the rest of the cell and therefore silver solution flows away toward the edge of the plate, consequently, we shall assume that the dark, irregular endothelial lines are precipitates of albuminate of silver, and darkened protoplasmic anastomotic processes, and therefore that they represent the outline of the peritoneal endothelia. It might appear that there is a wise provision in this interendothelial space, bathed in semi-

fluid or fluids to accommodate motion and function to such a degree that the endothelial plate will not be torn. It also allows space for the protoplasm of the cell to adjust itself to environments like soft clay. The brownish precipitates on the endothelial plate after the application of silver solution must be of an albuminous nature or due to young growing projecting processes of protoplasm.

In the interendothelial space is the seat of the physiology of the peritoneum. At the common junction of several (3 to 14) endothelial plates may be observed an oval or round opening known as a stomata. The mouth of this opening takes on a deeper stain than the surrounding endothelial plates, and it is lined by granular polyhedral cells. Some call them guard cells and their contraction and expansion regulate the size of the space; perhaps cilia may be found on them, as is asserted by some. The opening shows itself to have a depth, and hence may be termed a vertical canal. The interpretation is that the stomata (stigmata) vera are vertical canals lined by granular polyhedral cells and serve as a communication between the peritoneal cavity and the subperitoneal lymph channels. It seems to me that they regulate serious fluids.

Again, on the single interendothelial lines are found black dots, stomata spuria, or pseudo stomata, which are interpreted as connective tissue corpuscles or lymphoid corpuscles, in other words, as leucocytes passing into the peritoneal cavity. It is supposed that the young connective tissue corpuscle projects upward between the endothelial plates and becomes stained with the  $\text{Ag. NO}_3$  solution. Again, in the preparation of peritoneal endothelia with silver salts, we notice that portions of the membrane take on coloring of the silver much more intense than the adjacent portions. The intensely browned portion with very irregularly sized cells is interpreted as young endothelia or germinating endothelia. We also note after preparation of some portions of the endothelia that certain cells are vacuolating, i. e., the endothelia are multiplying to produce lymph channels. If the endothelia be stained with logwood, we note round or oval sharply defined bodies which are interpreted as nuclei. Thus in the technique of preparation of peritoneal endothelia, much interpretation lies in the kind of reagent employed. The technique of microscopical preparations of peritoneal endothelia and the interpretation thereof are neither universally agreed upon. Many claim that the interpretation of structures known as stomata vera rest on a faulty technique.

I wish here to speak of investigations made on the interendothelial substance in mammals, especially with osmic acid,  $\text{Ag. NO}_3$  and tannic acid. The employment of the osmic acid and tannin came from the suggestions of an article by Kolossow.



To this article I refer the reader for some excellent labors. The idea in the use of osmic acid is to produce fixation of the parts so that they can be studied at leisure. All the fine structures of the specimen can be seen in their natural relations. The second idea is the study of the parts properly colored. One can differentiate the various parts and their natural relations may also be viewed. The best microscopic results come then from a reagent which fixes structures in normal rela-



FIG. 40—(Author.) Rabbit's mesentery of small intestines. (Oc. 1, ob. 8a R.) This cut shows a tract of germinating cells (dark) joining ordinary endothelia cells (light); 1 to 1 nuclei, of both dark and light endothelial cells. 2, 2, 2, 2, light (old) endothelial cells. The old shades likely refer to age of endothelia. This cut is but a very small part of two large tracts dark and light of shade. 3, 3, dark (new) germinating endothelium. Observe how clear cut and prominent the nuclei are, though not stained with logwood. 4 and 5, clefts in endothelial plate.



FIG. 41—(Author.) Horse's mesentery. This group represents a typical one among innumerable others. 1, in the stomaverum is a much lighter and 2 is much darker colored granular cell; many small black dots exist (closed stomata). The endothelia are very regular, as is seen in the figure. This figure is carefully drawn after Oc. 4, ob. 3, (Reichert) and is quite true to nature in its general outline. 4, 5, 6, quite uniformly shaped endothelia, as may be seen over square feet of mesentery. The omentum of a horse is a very different appearing matter. It is a panorama of changing condition, germination, vacuolation, and varied shaped cells belong to it.

tions, and second, one which colors well the fixed parts. Kolossow prepared the following mixture.

Water distill. . . . .	45	cc
Alcohol, 85 per cent. . . . .	10	cc
Glycerine . . . . .	50	cc
Tannin Puriss. . . . .	30	grs
Pyrogallic acid. . . . .	30	grs
This mixture he calls a developer (entwickler).		



A second solution he gives as follows, viz.:

Alcohol, absolute.....	50 cc
Water distill.....	50 cc
Acid Nitricum Conc.....	2 cc
Osmic acid.....	$\frac{1}{2}$ gr

In the examinations of the peritoneal specimens the best results were secured when the osmic acid solution was combined with solutions of Ag. NO<sub>3</sub>. It requires many trials to perfect a single method. However, I secured very clear and elegant specimens by the following process:

1. The peritoneal specimen to be examined (perfectly fresh, if possible-), is washed with distilled water (some make it a .6 per cent. Na.Cl. solution).

2. The specimen is then put for five minutes in a 1 per cent. solution of osmic acid and  $\frac{1}{2}$  per cent. solution of Ag. NO<sub>3</sub>, equal volumes.

3. The specimen is then washed and put in the 2 per cent. solution of osmic acid for fifteen minutes.

4. It is then washed in distilled water and put in a 10 per cent. solution of tannin, with one part of glycerine added, for fifteen minutes.

5. Kolossoff directs that it is best now placed in a  $\frac{1}{4}$  per cent. solution of osmic acid for five minutes, when it is ready for preservation in alcohol.

The above method has produced in my work excellent fixation and beautiful coloring of specimens. On the specimens of the peritoneum produced by the combined action of osmic acid, Ag. NO<sub>3</sub> and tannin I have based my chief interpretations of the interendothelial space. It is a slow and tedious process, often accompanied by partial failures, but it produces in my opinion the most perfect fixation of the normal structures and the best coloring of the parts of any method so far suggested. We employed the peritoneal membrane with the above reagents in two conditions.

1. In the normal fresh state with as little trauma as possible.

2. After the peritoneal membrane had been stretched in various degrees.

If the peritoneum of an animal be selected, say a rabbit or a frog, in a fresh state and treated with due precaution from trauma by the reagents osmic acid, Ag. NO<sub>3</sub> and tannin, and mounted for search by an oil immersion lens, it will be clearly perceived that the interendothelial space is a complex matter. The interendothelial line dissolves itself easily into two lines with a clear space between them, and this clear space is crossed at various intervals by fine black lines.

The best comparison I can make of the interendothelial line is that it resembles a railroad with its ties. The two dark lines noted on the exact edge of the adjoining endothelial plates represent the two rails and

the dark transverse anastomotic protoplasmic processes represent the ties. By careful examination of the specimens and changing the microscopic focus it can readily be seen that the dark transverse anastomotic protoplasmic processes are exceedingly fine toward the surface but increase in thickness as they descend. With a very high power of oil immersion the single interendothelial line not only widens into two well defined lines with dark, well defined transverse protoplasmic processes, but one

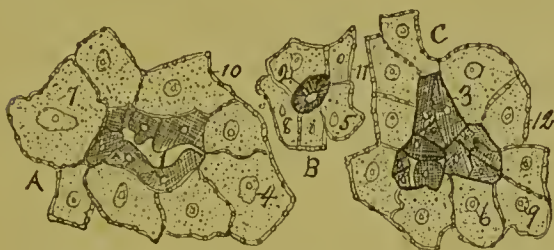


FIG. 42—(Author.) A, B, C, drawn from a shy-poke's peritoneum to show the variety of stomata vera. It may be remarked that the shy-poke's (bird's) peritoneum resembles the amphibia. The bird's ligamenti peritonei have less muscular elements. But the arrangement of the endothelia and stomata in birds resembles very much that of amphibia. This would be according to evolutionary processes. 1, 2, 3, stomata surrounded by granular, polyhedral nucleated cells, guard cells. 4, 5, 6, nuclei; 7, 8, 9, endothelia; 10, 11, 12, interendothelial space with its rings transverse anastomotic processes. It is in the interendothelial space of birds and amphibia where the essential analogy appears, as in both it is wide and distinct and suggested to me comparison of it to a railroad track with its rails and ties. Aves and amphibia resemble each other in the peritoneum, especially in the breeding season when some of the endothelial cells become partially ciliated and especially in the peritoneum of the ova sac of each genus, the connective tissue corpuscle which aids in forming the wall of the blood vessel is highly pigmented, presenting a beautiful outline of the blood vessel. It appears also that pigment is deposited in the interendothelial space. B. Drawn under 1-5 oil immersion lens. The shy-poke from which these specimens were taken was about 3 feet from tip to tip of wings.



FIG. 43—(Author.) From horse's mesentery. 1 and 2 point to a stoma vera in which 3 and 4 are protoplasmic spouts. 3 contains two or three nuclei and 4 contains one at least and may be two. They are in a state of rapid growth. 3 and 4 grow entirely above the common surface endothelia 5, 6, 7. 1, 2, 3 and 4 project so much above the surface that a fold change is required to see the surface and endothelia and the germinal endothelia. 1 and 2 are vacuolating, especially 2. This figure is adjacent to and surrounded by changes similar to itself.

may note that there is a central fine line of junction. But this middle line of junction is quite difficult of observation. The whole of the interendothelial line and space may be interrupted at irregular points by dark spots, rings with clear spaces or irregular dark masses. These dark masses (stomata spuria) on the interendothelial line are irregular in size and in distribution were called first by Virchow lymphoid cells; later by Oedmansson, connective tissue corpuscles jutting upward between the endothelial cells along the interendothelial line. This subject

will again be considered. Also in the normal peritoneum, not disturbed by trauma, there exists at the common junction of (3 to 14) endothelial cells an opening surrounded by small, polyhedral granular cells. These openings were discovered by Von Recklinghausen (1861), and termed stomata vera. The stomata vera are called by Klein centers of reproduction of endothelial cells, and the cells surrounding the stomata vera are named leucocytes by Ranvier. Others consider the stomata vera as channels which regulate peritoneal fluids. To this subject we will again return. Having now noted the normal appearance of the normal interendothelial line with definite reagents, we will attempt to further unravel the subject by experimenting with trauma on the peritoneum. The methods of stretching may be done by dragging on the part examined by natural filling of the gut in the animal during life or by distending the bowel or mesenterium by air as especially practiced by Kolossow. The interendothelial line shows a very different aspect if it be taken from a non-traumatized location or a place of absolute quietude, or whether it be taken from a place that has been subject to trauma, distension or stretching.

If a specimen of peritoneum be examined, be stretched or distended and treated carefully with the above reagents, osmic acid to fix it and Ag. NO<sub>3</sub> to darken the interendothelial lines and tannin with glycerine to endow it with a peculiar rich, bluish black color, we will observe under the microscope with the oil immersion the following: The interendothelial line has been enormously broadened. The transverse anastomotic protoplasmic processes are wider apart and much thickened. Round, oval or elongated rings with spaces are plainly visible along the interendothelial lines. The anastomotic, transverse protoplasmic processes are distinctly finer toward the surface and distinctly thicker as they descend from the surface. The upper edge of the cover-plate does not show anastomosis with its fellow, only the protoplasm of the cell manifests the dark connecting fibers. The stretching of the peritoneum may show a line of dark rings for the interendothelial lines, like a rosary or a string of beads. But oftener the interendothelial line, after trauma, stretching, distension and so forth, is an irregular one. The dark rings with clear spaces are quite irregular. Some are large, round, oval or elongated. Some points are simply a black mass with no central clear space. The stomata vera and stomata spuria are disturbed and distorted so that all relations are altered. However, repeated examinations of specimens, after trauma with the same reagents, produce similar appearance. We observe then that the interendothelial space of the peritoneum under reagents present three objects for examination, viz.: (a) double interendothelial line with its transverse processes connecting the protoplasm of the cells. (b) The stomata vera



situated at the common junction of the interendothelial plates. They are irregular in number and distribution. (c) The stomata spuria, situated on the interendothelial line. They are irregular in number and distribution.

What is the nature of the endothelial line made manifest by the action of  $\text{Ag. NO}_3$ ? It is one dark line under low power. Under high power it shows itself to be two distinct parallel lines with dark trans-



FIG. 4.—(Author.) Omentum of woman of thirty. Died twenty-four hours after delivery, of puerperal eclampsia. No peritonitis. (Oc. ob. 8a, R.). 1 points to a stoma verum. The growth process seemed to be going on under the endothelia. 9, stomata with contracted protoplasm; 2 and 3, halves of stomata vera; 5, large nucleus, and 4, other smaller ones in shimmering through the endothelial plates. Many similar adjacent figures may be observed in the microscopic field. In cell 1 may be seen dark half-moon-shaped clumps with many glistening nucleoli. In 3 may be seen glittering points existing. It appears that cells 6 and 7 are in a process of division. 7 is brown and 6 is light. This figure I sketched with much care to resemble as nearly as possible the microscopical appearance of one stoma verum and its group of endothelia surrounding it.

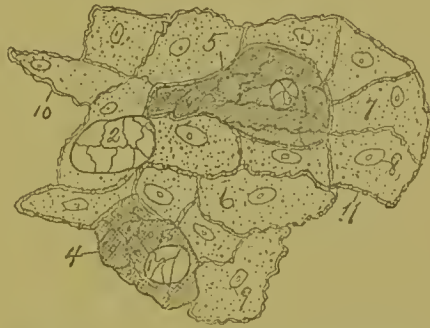


FIG. 45.—(Author.) Drawn from gastrosplenic omentum of gopher. 4 and 5 are germinating endothelia building over the common endothelia. In each band composed of small, young nucleated cells, there is a developing sinus, 1 and 3. At the point where the bud 5 sprouts, (doubtless a stomum) is another lymph sinus, 2. 6 and 7, common endothelia. 8 and 9, nuclei. The building endothelia stains intensely with  $\text{Ag. NO}_3$ . 10 and 11 point to the interendothelial space with its numerous rings or rather transverse processes and its two parallel lines. The reason rings appear is because the transverse process is thick at the base and thin in the center. The gopher's peritoneum is exceedingly thin.

verse connecting processes. Under very high power it resembles a railway with its ties. Because it is dark after the reagent  $\text{Ag. NO}_3$ , it is considered to be an albuminate of silver. Some investigators consider the interendothelial substance as a kind of cement, others, a thick fluid or semi-fluid substance. One writer, Robinski, denies the existence of the interendothelial substance altogether. Klein says Robinski's assertion is not worth consideration, or, more accurately, unnecessary to discuss. But according to my own investigations of the interendothelial substance, Robinski's conclusions seem more probable. In my earlier



microscopic investigations of the interendothelial space, his view appeared untenable, but the more carefully I studied specimens, especially with osmic acid, Ag. NO<sub>3</sub> and tannin, the more tenable appears his idea that there is no special interendothelial substance, but that the appearance under the reagents Ag. NO<sub>3</sub> is merely the precipitation of a superficial albuminous fluid.

When we carefully study well prepared specimens with accurate coloration we really do not see simply a dark interendothelial line, but a network or chain of anastomosing protoplasmic processes. The more trauma the peritoneal membrane suffers, the more the reagents bring out interendothelial network of connecting processes of the cells. Besides, by separation of the endothelial plates, the interendothelial rings enlarge in size and thickness, all of which does not act like a cement substance but like a network of anastomotic processes. In my labors the more I stretched the peritoneum the wider was the interendothelial network and the larger were the interendothelial rings.

Kolosow showed that the more he distended the peritoneum over a bowel with air the wider was the interendothelial meshwork of rings and anastomotic process connecting the cells. Microscopical labors with new reagents have demonstrated that the endothelial cells are not single, isolated masses of protoplasm, but they are connected to the adjacent cells by distinct radiating transverse projections of matter which are rendered black with Ag. NO<sub>3</sub>. This makes every cell a member of a colony of cells, a complement of every other cell. Kolosow followed out an old view, first advanced by Bichat and confirmed by Ranvier and later revived by Muscatello, to the effect that the mesenterium can be distended by placing a tube between its layers and forcing in air. The mesenterium will not only distend but will remain distended some time, due, doubtless, to the membrana limitans not being perforated in the mesenterium. By distending the mesenterium gradually to various degrees Kolosow secured an interendothelial network proportional in width to the degree of distension. I secured similar interendothelial figures by taking the peritoneum from a distending and contracting bowel or irregular ones by stretching the peritoneal membranes. Is the generally accepted opinion correct that the interendothelial substance is fluid, semi-fluid or a kind of cement substance? Washing the peritoneum in distilled water does not prevent the interendothelial lines from arising on the application of Ag. NO<sub>3</sub>. If it were a fluid the interendothelial lines should be of uniform breadth on distending the peritoneum. This, however, is not the case as we can note very irregular breadths, showing that some parts in the interendothelial line yielded to the force more than others. The transverse connecting processes yielded more at some points than at others, all of which can

be observed in specimens. If it were a fluid substance, by separating the endothelial cells and then applying the Ag. NO<sub>3</sub> the interendothelial line should be correspondingly wide, a broad stripe, and not a fine, single line as under low power. But this is not the case, for the wider the interendothelial line the more it is a network, not a single broad line, but two lines. If the cells are held together with a semi-fluid substance or by organized protoplasmic processes, there will certainly be a difference in appearance when the cells are separated from



FIG. 46—(Author.) Is a portion of a young rabbit's omentum close to the pylorus with its endothelium brushed away and stained with  $\frac{1}{2}$  per cent. solution of silver nitrate; also with logwood. It shows a spot in the omentum (no doubt over a wide lymph channel) with numerous stomata vera, both open and shut, also stomata spuria. The endothelia and nuclei are plain. I sketched it carefully under one field of the microscope of about 400 diameters. Any portion of this rabbit's omenta presents elegant views of stomata. Only few stomata spuria (S.S.) appear in the field, as no doubt this field is over a wide lymph channel. C.S., closed stomata vera; N., nucleus; S.V., stoma verum.



FIG. 47—(Author.) Peritoneum of fish (Oc. 2, ob. 8a.), taken from a carp about  $3\frac{1}{2}$  inches long. Ag. NO<sub>3</sub>,  $\frac{1}{2}$  per cent. applied. 1, 2, 3, 4, 5, represent stomata vera of very similar shape and appearance to that found on the diaphragm of rabbit and man. 6, 7, 8, 9, nuclei; 10, 11, common endothelia. The peritoneum of fish over the bladder, from which place the specimen was taken, shows very numerous stomata vera. Common endothelium with two nuclei. The interendothelial line under high power divides into two parallel lines with transverse anastomosing processes. The endothelia of the fish are very irregular in shape and arrangement. 13, 14, 15, indicate the interendothelial space with its two parallel lines and transverse process (railway track).

each other by force. Fluid and organized processes do not separate alike under like circumstances. It will surely require more power to separate an organized connection than fluid connection. Well prepared peritoneal membrane under the oil immersion shows, instead of the broad black band which must arise if the interendothelial line be fluid or semi-fluid, a peculiar network of processes of fine lines. It looks like a chain. Some might compare the interendothelial space to apertures, rings and dark spots. The comparatively wide interendothelial space is crossed transversely and obliquely by fine black lines which extend

from one protoplasmic portion of the cell to its adjacent fellow. The portion of the interendothelial space not filled in by black lines is perfectly clear and transparent, i. e., composed of a thin layer of protoplasm. In many cases, no doubt, it is simply the membrana limitans, a glass-like membrane, which we observe.

The interendothelial space appears sometimes as if it were filled in by peculiar rings which have unequal thickness in their circumference. The thinnest part of the rings lies nearest the surface and the thickest portion lies below the surface. At other times the interendothelial space resembles a rosary or string of beads, or a number of coins arranged in a flat row, and the clear spaces may be uniform or non-uniform. The width of the transverse protoplasmic processes varies very much in size. There may be a ring with a broad black circumference and clear white internal space, or there may be simply a black flat space. The size and numbers of the rings or transverse processes in the interendothelial space vary in direct proportion to the amount of distension or dragging on the peritoneum. I have repeatedly observed that by slight trauma the interendothelial space is not very manifest, but by gradually increasing the trauma on the peritoneal membrane the interendothelial space increases in its rings, black spots, transverse processes, its network, and especially the white portion of the interendothelial space. This experience agrees entirely with Dr. A. Kolossow, who spent two years in the laboratory of the late Prof. Babuchin at Charkow, Russia, studying the endothelia. Dr. Kolossow, now professor of histology in the Imperial University of Moscow, Russia, revised his laboratory labors and published them in the *Archiv fur Mikros. Anat.* in the German tongue.

The network of transverse connecting processes in the interendothelial space does not depend on the silver stains, but can be produced by other methods. Previously washing the endothelial membrane does not hinder the arising of the interendothelial network. However, washing does not hinder irregular precipitated deposits on the interendothelial plate itself; nor does it change the number of stigmata or stomata nor alter the character of the interendothelial structure.

E. Klein, of London, England, to whose excellent work I am indebted, says: "There exists a number of facts which show that the existence of the silver lines (i. e., interendothelial) depends also and chiefly on precipitations in an intercellular albuminous substance which holds together the individual endothelial cells." It is quite evident that Klein believes that the endothelial cells are held together by an albuminous substance and not an organized system of anastomotic processes. Other authors consider the black interendothelial lines as the result of the precipitation of a serous fluid lying between the cells.



Again, others say that the black lines on the peritoneal membrane produced by Ag.  $\text{NO}_3$  are due to the precipitation of an albuminate fluid lying in the furrows on the endothelial plates. But no one doubts today, so far as I am aware, that the dark lines from Ag.  $\text{NO}_3$  solution on a peritoneal membrane is the expression of the outline of the endothelial cell.

In 1880 Klein, in his *Atlas of Histology*, again asserts that the endothelia are held together by a semi-fluid albuminous cement substance, not changing his views from a seven years previous publication.



FIG. 48—(Author.) Drawn from the gall-bladder of a toad. 1, 2, 3, large pale nuclei; 4, 5, endothelia, which are characteristic of the shape of endothelia situated on very expansive and contracted hollow organs, i. e. rounded and irregular borders. 6 and 7 point to the interendothelial lines, which are very narrow in this specimen. Under 1-15 oil immersion lens it dissolves into two very closely parallel lines, with a row of bead-like white spots situated between them. The cover-plates are immeasurably thin.

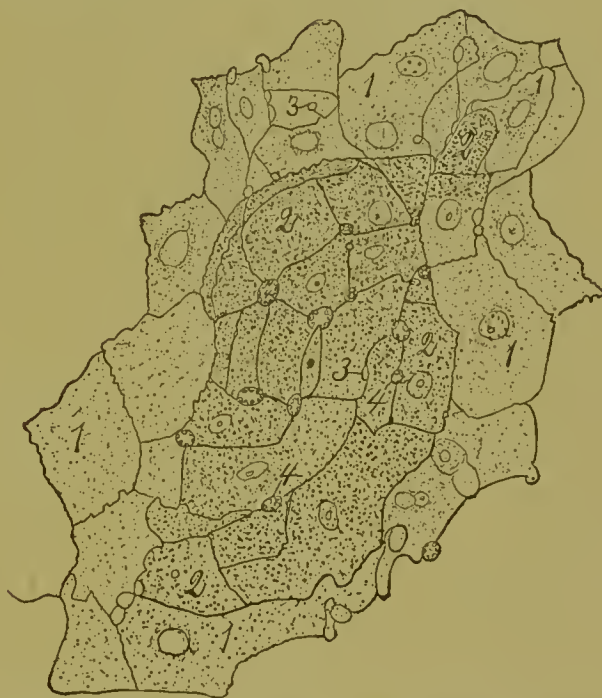


FIG. 49—(Author.) Dog's gastro-splenic omentum. Three months old. (Oc. 2, ob. 8a, R.). Carefully drawn and shaded to show the area of germinal endothelium. 1, 1, 1, 1, shows the old non-granular endothelial cells; 2, 2, 2, 2, shows the endothelia granular, new, germinating. It grows in patches; 4, 4, 4, stomata vera; 3, 3, stomata spuria. Observe the distinct outline of the darkly shaded growing patch of endothelia growing over the surface of the old endothelia which is shaded light. This is a non-fenestrated new part. Large numbers of patches are growing adjacent to this.

Muscatello, a devoted disciple of Bizzozzero, in an excellent article in *Virchow's Archives*, 1895, discards the semi-fluid or fluid cement substance between the endothelial cells and substitutes organized protoplasmic processes, incidentally remarking that it is in general a law that all cells are connected by organized processes. Pflueger, Heitzmann and recent writers have generalized the views that cells are connected into colonies by projecting processes of protoplasm.

Hence, our observations of specimens have led us to the view that there is no interendothelial fluid, semi-fluid or cement substance; but that the interendothelial network of transverse lines, white or clear spaces, rings, etcetera, represent an organized connection between the protoplasmic portions of the endothelial cell. The cover-plate shows scarcely any anastomosis with its fellow—perhaps a few slight, fine processes may be observed projecting from the lower edge of its border. There is no doubt, however, that the endothelial connection is a complicated process, and without appropriate reagents little may be expected in the way of discovering its ultimate structure. With the osmic acid, Ag. NO<sub>3</sub> and reduction with tannin one can observe that the surface portion of the endothelial cell is an indurated or hardened smooth plate, and that it does not engage to any great extent in the connecting anastomotic protoplasmic processes which hold the adjacent endothelia together as a membrane. These reagents show the protoplasm of the cells to be connected to each other by fine processes. It is probable that there is no hypothetic cement substance between the endothelial cells; the reason that the dark lines appear after Ag. NO<sub>3</sub> is applied is that a superficial thin layer of albuminous fluid bathes the interendothelial space. The cover-plate is really not connected with its fellows. It merely rests in contact with them, i.e., just touches them, with scarcely an anastomotic connection, or at least a very superficial anastomosis. The cover-plate is an acquisition to the cell from evolutionary processes from friction and pressure while the lower or protoplasmic portion of the cell is connected to its fellows by numerous fine, anastomotic protoplasmic processes. The anastomosis of the endothelial cells increases in strength from the surface downward. The anastomotic processes are finer and shorter toward the surface and thicker and longer as they descend from the surface. Hence the appearance of rings and oval which have a thicker portion at one part than another, i.e., the lower segment is the thicker. Some writers speak of very long anastomotic processes such as reach from one cell across beneath the adjacent cell. I have never been fortunate enough to see these very long processes. Kolossov speaks of a process by which the anastomosis may be seen very plainly between the endothelial cells. It is based on the well recognized principle that the endothelial cell is elastic and contractible. If the peritoneal membrane be allowed to dry some fifteen minutes before it is fixed by osmic acid and reduced by tannin, the resulting specimen shows the anastomotic processes much more manifest, longer than otherwise. The drying of the endothelia induces the protoplasm of the cell to contract and thus the anastomotic processes, if not ruptured, must be more exposed and hence appear longer. It may well be remembered that the combined cover-plate and the protoplasmic portion

of the endothelial cell present but a very thin plate and vary in the same and different animals. However, when one brushes off the whole endothelial cell it leaves pits or depressions on the membrana limitans. The typical interendothelial space with its lines, rings and stomata are to be looked for in the amphibia, especially the frog and turtle. In the ascending scale of animal life the interendothelial space is not so markedly manifest. Yet appropriate employment of reagents and oil immersion lens reveals the interendothelial space well in all animals examined.

Finally, the connection between the endothelial cells produced by an-



FIG. 50—(After Peter Nikolsky, 1880.) Represents a stoma verum of four cells surrounded by some twenty radiating endothelial cells. Drawn from the mesentery of a male frog. Few could doubt that this is a primordial arrangement.

astomotic protoplasmic processes excludes the old, commonly accepted views of a fluid or semi-fluid cement substance. The reason is that the dark interendothelial lines do not lie between the endothelial cells but precisely on the borders of the cover-plate, the real connection of the cells being deeper down in the protoplasm of the cells. It is very apparent with a well prepared specimen of peritoneum with osmic acid, Ag.  $\text{NO}_3$  and tannin and an oil immersion lens that the interendothelial line which by low power is only one line, resolves itself into two distinct lines lying parallel to each other and with a clear space between them. But each line is exactly and precisely located at the extreme edge of



the cover-plate, in fact, it is the edge of the cover-plate itself, and not in the lower protoplasm. There is then but a slight basis for the argument that there exists cement substance between the cells when the modern microscope and reagent have dissolved the erroneous opinion. The oil immersion with the new reagent demonstrates definitely that the real anastomosis of the endothelial cell is in the protoplasm embracing the nucleus beneath the cover-plate. It cannot be denied that in the living animal the interendothelial space is bathed with an albuminous fluid which precipitates on the application of  $\text{Ag. NO}_3$ . The interendothelial space may be filled with lymph containing albumen. Now, the albuminous fluid may be in a very thin layer and is promptly precipitated by  $\text{Ag. NO}_3$ , which, after it has precipitated the albumen on the surface, forms a distinct barrier to any further progress of this reagent. The silver salt cannot penetrate beyond the insoluble albuminate layer it has itself made. But if one allows the silver salt to remain long in contact and under long continued sunlight with the specimen, it gradually penetrates further, making broader brown lines and stripes. In the specimen it is a striking feature to observe a black silver line exactly along the border of each cover-plate with the white endothelial space crossed by the fine dark transverse lines. Some very elegant specimens may be obtained to fit the above description from the hollow organs which expand and contract as the gall-bladder, especially the stomach and small intestines. The frog's mesogaster is one of the best portions of the peritoneum to show well the interendothelial space with its intricate meshwork. The hollow organs which expand and contract or what would be the same, distension by air, gives splendid specimens, for the cover-plates are considerably separated from each other, allowing the osmic acid to fix the network and the  $\text{Ag. NO}_3$  solution penetrates deep into the interendothelial space, blackening all the parts bathed by a thin layer of albuminous fluid. Doubtless this is the reason that we find here other black flecks or discs. At such a point a depression or pit in the interendothelial space may be bathed with albuminous fluid in such a manner that the silver solution flows over the whole surface and all is blackened. Also the spot may be a black ring with a clear center. This clear center may be a leucocyte passing into the peritoneal cavity and the black color is the precipitation by the silver of the thin fluid albuminous layer resting on the leucocyte's surface. Also by forcible separation of the cover-plates tissue (protoplasm) will be bared, exposed on whose surface lymph or albuminous fluid will ooze and become precipitant by the silver solution producing a black disc. Almost all shapes of rings, oval, discs and oblique bars may be produced by trauma on the peritoneal membrane by irregular forces producing irregular exposed patches which are soon bathed by lymph in the inter-

endothelial spaces, and on application of silver solution various black figures arise. It is only natural that lymph will immediately ooze out on a freshly exposed tissue made so by recent trauma. Besides, it is likely that a thin albuminous fluid bathes the interendothelial space constantly, i. e., this fluid covers the protoplasmic anastomotic processes.

But, no doubt that during the operation of separation of the interendothelial cells and their cover-plates many anastomotic processes are ruptured, allowing exposure of their ends bathed in albuminous fluid to the silver solutions and such ruptured ends may produce some of the irregular figures from the silver stain, as a freshly ruptured or traumatized protoplasmic process is sure to be bathed with a fresh, thin layer of albuminous fluid.

Again, we must not forget that the interendothelial processes possess

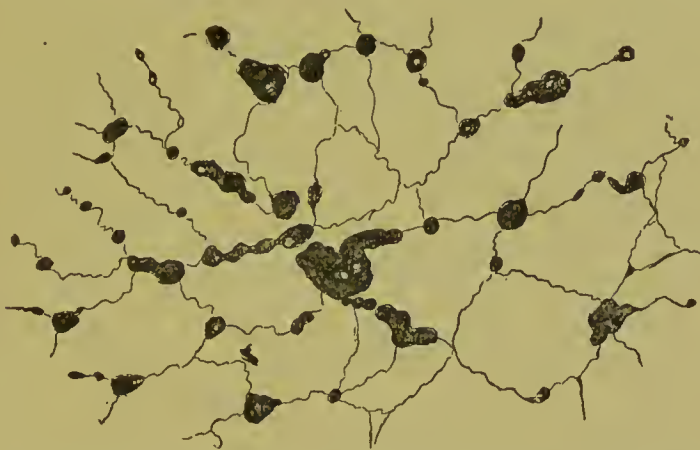


FIG. 51—(After Oedmansson, 1863.) Mesenterium of frog. Between the cells lie a number of larger and smaller colored (black) points of various forms and sizes. Silvered roundish openings lie at the common junction of several cells as well as on the interstitial space. Silvered at the upper left hand corner is a drawing of rabbit's omentum. Behind the black point, lying at the common junction of the cells, one can see the round outline of a cell. At the lower left hand corner is a drawing of the endothelial plates of a rabbit's omentum. At the common junction of the endothelial plates lies a cell with a nucleus.

a great power to contract and expand but especially to contract, which power must make a vast difference in the width of the interendothelial space. Especially is this true when irritating substances enter the peritoneal cavity. The sudden contraction of the protoplasm of the cell from the irritation so widens the interendothelial space that it allows the entrance of the irritating (infective) matter through the insufficiently guarded parts of the interendothelial space. Doubtless the sudden contraction of the protoplasmic portion of the endothelial cell from irritation (infection) explains its rupture and separation from adjacent cells, and finally its separation from the ground substance as in peritonitis. The cover-plate, not being as contractible as the protoplasmic portion of the cell itself, soon becomes separated and floats away in

the fluid usually existing. Dr. Kolossow goes so far as to call the openings in the intercellular space canals or tunnels.

The subject of interendothelial space is all important in the study of the free peritoneal surface, as it appears to me the chief physiology of the peritoneum lies in the interendothelial space. We noted above that the chief anatomical structure was located at the common junction of several endothelial cells, stomata vera, and it appears that the chief physiologic function occurs through the means of this structure, i. e., the regulation of fluid currents and to produce new endothelia.

Now, besides stomata vera and spuria there is a large interendothelial space filled with matter which stains dark and brown with  $\text{Ag. NO}_3$ . The precipitate produced by the  $\text{Ag. NO}_3$  solution of  $\frac{1}{4}$  to  $\frac{1}{2}$  per cent. is considered to be an albuminate of silver. The precipitate arises slowly in fresh specimens and gradually increases in breadth from a fine, dark, irregular line, just perceptible with a high microscopic power, to a broad line extending even over the surface of the adjacent endothelial plate. The center of the line one might say is black, but the color of the line becomes brown as it recedes toward the nucleus, i. e., precipitated material on the endothelial plate is nearly always brown. It appears from the use of  $\text{Ag. NO}_3$  solution on the peritoneal endothelium that the endothelial line may be announced as black, while that on the surface of the plate is brown. Whether this is due to two different kinds of material, one for the surface of the plate and one for the interendothelial space on which the silver solution acts differently, or whether it is due to the quantity being more in one place than another is still uncertain. It may be that a thin layer of albuminous substance appears brown and a thicker one dark. It may also be thought that the age of the material on which the  $\text{Ag. NO}_3$  solution acts is different. The matter on the surface of the endothelial plate may be older than the interendothelial matter. However, the interendothelial substance was considered to be a soft, semi-fluid material of an albuminous nature by older authors. If so it is pliable and yieldable, allowing considerable motion to an endothelial cell without destroying its integrity of position or function. It adapts the cells to strains and trauma without rupture. Really the endothelial cells are resting in a semi-fluid bed and the interendothelial substance acts like a buffer in sudden motion. It endows the endothelia with power to suddenly alter their course, position and relation without losing the integrity of structure and function. This interendothelial semi-fluid bed adapts itself readily to sudden changes as in the filling and emptying of organs and vessels. In an over-filled bowel the peritoneal layer gives way first. I have proven that peritoneal rents occur first in over-filling of dog's intestines showing that the interendothelial space is put to active service in dilatation and contraction of



organs and vessels. The interendothelial space shares in inflammatory attacks for the endothelia desquamate, leaving only the pitted semi-fluid bed which can illy protect itself because its fluid nature absorbs the invading hosts. The endothelial plates are so adjustable and the interendothelial line is of such a magnitude that the endothelial plate has a considerable range of normal adaptability. To the anatomic and physiologic structures (stomata vera and spuria) found in the interendothelial semi-fluid albuminous bed must be looked for new revelations in further research, which must be of an experimental order. If one places peri-

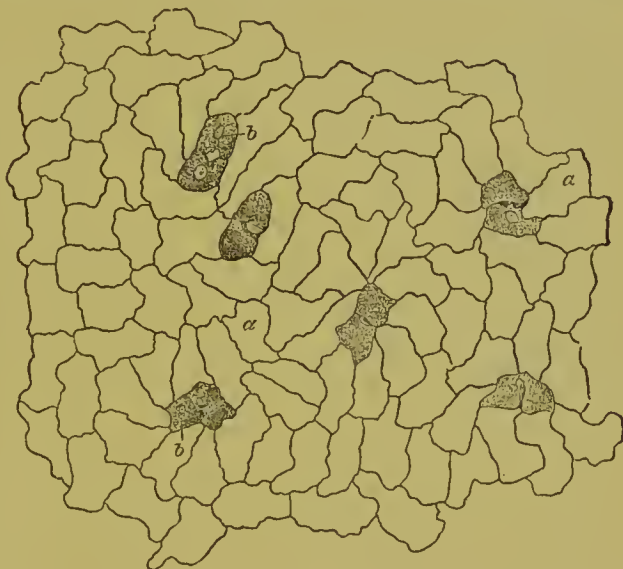


FIG. 52—(After Handbook for Phys. Lab., Vol. II., 1873.) Mesentery of the frog colored in silver. (a) Ordinary surface endothelium. (b) Endothelial cell surrounding a simple true stomata. These cells have the germinating character, are distinctly granular, and are not flat like those which surround them. (Oc. 4, obj. 5. Tube not drawn out.) This specimen illustrates 6 typical stomata vera.

toneum in water and allows it to remain several hours the interendothelial line appears to enlarge, i. e., it appears to swell by absorption of water, and the reverse is true if one places the peritoneum in alcohol or formaline. (In regard to the interendothelial substance, I wrote some of the above when I believed in this cement substance, but with more investigations and the use of more perfect reagents I gave up the interendothelial substance and replaced it by the term interendothelial space. I leave the above paragraph as it was written to show the old and new argument.)

In conclusion regarding the interendothelial space we would note :

1. There is no interendothelial cement substance of a semi-fluid character, but consists of anastomotic protoplasmic processes bathed by a thin layer of albuminous fluid which precipitates dark with  $\text{Ag. NO}_3$ .
2. The cover-plate or superficial non-nucleated portion of the endo-

thelial cell rests only in contact with its fellow and has no organized connection with adjacent endothelial plates unless it be a small portion of the inferior edge.

3. The lower portion of the endothelial cell, the real living protoplasm containing the nucleus, is connected to its adjacent fellows by organized protoplasmic processes which anastomose with corresponding processes of adjacent cells, forming cell colonies.

4. The name interendothelial substance of previous authors we would displace by the term interendothelial space.

5. The interendothelial space is a net or meshwork of protoplasmic organized processes, which being bathed with a thin layer of lymph, albuminous fluid, blackens on the application of the silver solution.

6. In the interendothelial space the network of protoplasmic processes increases in a downward direction in the progressive separation of the endothelial cell, i. e., the deeper one can see the more processes are visible. The separation of the endothelial cells may be due to trauma or irritating (infective) substances. The protoplasmic cell body under the cover-plate may contract and this will not only make the anastomotic processes appear larger, but also to appear thinner, and appearing thinner, more processes will show themselves in the field.

We will here consider the stomata vera, which are round or oval openings situated at the common junctions of (3 to 14) plates. They were discovered on the abdominal surface by Von Recklinghausen by means of injecting milk or other finely divided matter into the peritoneal cavity of animals and then tracing its absorption through the abdominal serosa by the aid of staining with silver solution. The careful methods of experiments by which Recklinghausen arrived at his conclusions in regard to the stomata vera on the diaphragmatic serosa are worthy of the highest admiration. With persistent and indefatigable labor he worked the matter out systematically from beginning to end, the chief part of which may be read in Virchow's Archives. The stomata vera are among the chief structures in the endothelia of the free surface of the peritoneum. They are found easily and distinctly in all the animals enumerated in this work. In fact, no animal was found without numerous stomata vera. The typical locality of the stomata vera are the omenta and abdominal serosa of the diaphragm. However, my best specimens generally come from the gastro-splenic omentum of frogs. They may be found open or closed. The embryonic pig, the rabbit and the frog furnish in my experience the most typical stomata vera. It appears to me that the stomata vera are the centers, or pre-formed openings, around which endothelia group themselves. The embryo pig on its gastro-splenic omentum furnished the most typical, sys-

tematic and numerous grouping of endothelia about stomata of all animals examined.

The cells which surround the stoma verum are polyhedral, club-shaped, and of a dark red granular appearance. They have an ovoid or spherical shape. There may be but two or there may be six or eight surrounding the stomata. The cells may be large as an adjacent flat endothelia plate or not be more than 1-15 or 1-20 as large. The especial distinction of the cells is that they are very granular. Some have claimed that they have found cilia on the cells lining the stomata vera.

If one examines the stomata vera, situated at the common junction of several cells, with a high power after staining with Ag. NO<sub>3</sub> solution, there can be observed at its mouth, which opens on the free surface of the peritoneal endothelium, small reddish granules possessing nuclei. These small masses are much darker red than the surrounding, and of

FIG. 53—(Author.) From hepatic ligament of hen, showing a very distinct stoma verum with six granular polyhedral cells and also to the right of the stoma verum a dark granular cell, not capable of division by high power. It appears to be a deviation from the stoma verum. There is a large amount of interendothelial substance in the hen's peritoneum and it is quite easily separable by high power into two dark lines with fine transverse dark lines. Also may be seen in the hen's peritoneum typical ciliary processes.



a distinctly granular character and may present a granular polyhedral shape with nuclei. They are in all probability young germinating cells lining the surface of the canal known as the stoma verum. The silver solution intensifies their color from possessing considerable precipitable albumen. After considerable time spent on examinations of many scores of specimens, I am convinced that the stomata vera are canals lined with granular polyhedral nucleated cells. The stomata vera are not only surface mouths but canals of more or less perceptible length. In short, they are vertical canals lined with granular cells passing through definite distances and structures of the peritoneum. In the embryo pig the stomata vera may appear like looking down a long, thick cylinder. It appears like the aperture of a well lined with stone. The stones may represent the granular cells. In the diaphragm of the rabbit I found some of the stomata vera canals passing obliquely from the peritoneal endothelia in the subserous lymph channels showing a distinct length. If trauma be inflicted on the specimen examined one may frequently observe a part of the granular polyhedral cells, which line the vertical canal adherent in a beaded line broken away and floating about in the glycerine under the cover glass. It shows distinctly where it was previously situated and waves about still as an intact portion of the cellular membrane lining the canal. Again, trauma may be



due to the Ag.  $\text{NO}_3$  solution which will produce a cleft between the lining membrane of the vertical canal or stoma verum and the smooth mouth made by the common junction of the endothelial plates. The cleft may be empty or filled with granular debris. The cleft or rift between the granular lining cells of the stomata verum and smooth circumference produced by the common junction of the several endothelial plates may be compared to the separation of the mucous membrane of the ureter from its outer wall produced by passing a sound. The loose mucosa of the stomach may also be compared to this traumatic separation. It is easy to separate the stomachic mucosa from the outer wall. It may be that the granular lining membrane of the vertical stomata vera contracts by the application of Ag.  $\text{NO}_3$  solution. The vertical canals of which stomata vera are the mouths are not always perpendicular to the peritoneal surface. They may pass from the endothelia of the free peritoneal surface to the subperitoneal lymph channels in an oblique or curved direction. The specimens obtained from a rabbit's diaphragm after injecting a carmine solution into its abdomen fourteen hours before death would indicate that many of the stomata of the lymph channels lie immediately beneath an endothelial plate of the peritoneal surface, and it would appear that occasionally the endothelial cells show marked symptoms of a very granular nature and a semi-fluid character. It is difficult and almost impossible to assert whether the granular condition of the peritoneal endothelia immediately over the stoma of the lymph endothelia is original or acquired. But when a stoma verum of the peritoneal endothelia is found directly over a stoma verum of the endothelia covering lymph channels, the picture changes to something more definite. By slowly turning the fine adjustment serew of the microscope one can view the interior of the vertical canal with its granular lining cells for some distance, especially the oblique canals. The most typical specimens for studying the relations of the stomata vera of the endothelia of the free peritoneal surface to the subserous lymph channels came from the serosa on the abdominal side of the diaphragm of a rabbit which had been injected with a solution of carmine the day before it was killed. The carmine had gained access to the subserous lymph channels and was impacted in the stomata of the endothelia covering the lymph vessels. The carmine made them easy to observe, through its red color. The frog shows the relations well in some specimens. The stomata vera may be found closed partially or completely or they may be found wide open. The frog and rabbit show typical specimens in regard to the degree of closure. Again, there is a condition of the stomata vera which has induced endless discussion and many interpretations. In short, it appears to be a condition where the stomata are filled with granular material like grains of corn-meal colored dark red

by silver salts. Is it not this condition that surely puzzles Muscatello? He concedes they are stomata, but a kind of automatic stomata which allow passages of finely divided matter when it really forces the granules apart, and slips through. He claims that such stomata vera are just like the apertures in the walls of blood vessels which every now and then allow the exit of a certain number of blood corpuscles, and again close. Of course, it is easy to recall similar conditions asserted by Recklinghausen when he said he could see whirls produced in the milk globules and see the milk globules duck under the peritoneal endothelia and disappear, but he never could find the real mouth which received

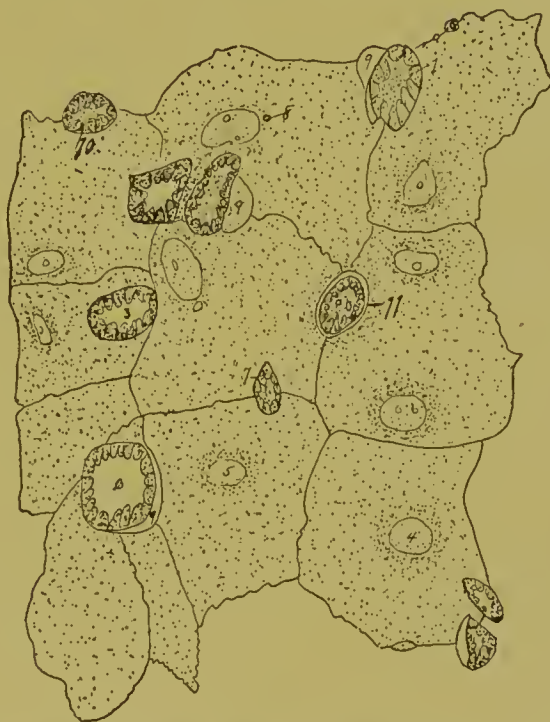


FIG. 54—(Author.) Rabbit's gastro-splenic omentum. (Oc. 4, ob. 8a., Reichert.) Ag.  $\text{NO}_3$ ,  $\frac{1}{2}$  per cent. applied. 1, 2, 3, stomata vera; 4, 5, 6, nuclei; 7, stomata vera (location?); 8, intraendothelial stomata; 9, rifts or clefts where the granular cells have retracted in the form alive. This figure was drawn with very high regard to nature as it appeared under the microscope. The stomata are a prominent feature here.

the milk globule until he marked the spot of the ducking under of the milk globule and then allowed Ag.  $\text{NO}_3$  to trickle under the cover glass whence by its stain it showed at the marked spot the stoma verum, but no open mouth. All that Recklinghausen could see was the dark granular cells marking the stoma verum which had opened to allow the milk globule to pass and closed again, leaving no trace of an open aperture. It is like a swimmer diving in water—he has left no aperture behind—all is closed. Those who have watched frogs in a pond during the summer season where the water was covered in places entirely by soft green vegetation have seen the frog dive through this vegetation into the

water beneath, but the springy, spongy vegetable matter closes immediately after the frog and no trace is left behind. This granular or fluid condition of the mouth of the vertical canal is not well understood. It may be that the canal has an elastic sphincter and that an excess of granular cells exists at its mouth.

What are the functions of the stomata vera? First, the endothelia immediately surrounding them stain darker red than that more distant, so far as we know, indicates newer cells or younger protoplasm or more perceptible albumen by Ag.  $\text{NO}_3$ . Hence, it would appear that the stomata vera are the source of new endothelia to supply the ranks of dying comrades. Second, it would appear from investigations that they were the regulators of peritoneal fluids. For example, if inflammation attacks the stomata vera, too much or too little fluid will prevail in the abdominal cavity. Inflammation of the granular cells which line the vertical canals—stomata vera—would enlarge them and obstruct the return flow of peritoneal fluid, resulting in ascites. The active condition of the stomata vera may account for the rapid death in perforative peritonitis by quickly absorbing the toxic matters existing in the peritoneum. The stomata vera, as anatomic and physiologic structures give, at least, a reasonable explanation of the existence and regulation of the peritoneal fluid. Ascites must, of course, rest on inflammation of the cells in the vertical canals or the endothelia. The claim that stomata vera only exist on the diaphragmatic serosa of the peritoneum must be emphatically denied. Dubar and Remy claim that matter in the peritoneal cavity is absorbed by other peritoneal regions than the diaphragm, and no doubt it is true. The claim that stomata vera do not exist as anatomic and physiologic structures is generally based on the idea that they are irregular in number and distribution; that large areas exist without a trace of them. Others claim that stomata vera are the result of Ag.  $\text{NO}_3$  or of some trauma, artificial products. They are simply the results of precipitation in the intercellular space. Muscatello, even, claims that they are the result of the endothelia separating and precipitation of the intercellular substance. He says the stomata vera are the same kind of openings which exist in the walls of the blood vessels that will, under certain circumstances, allow the escape of many white blood corpuscles and reclose. This comparison makes no denial of the existence, only it belittles the high significance of the stomata vera. At first it appeared that I was able to note some especial difference in endothelia and stomata in different animals to account for the difference in resistance to peritonitis, but so far no light has broken on that subject. For example, the mare has so slight resistance to peritonitis that it is not practical to perform laparotomy on her, and puerperal fever in the mare is rapidly fatal. So far as I am aware solipeds



do not resist peritonitis well. Resistance in man and in the dog is about equal, while the pig resists peritonitis to a high degree. Yet, so far the microscope has indicated to me no marked differences. Much stress is laid on the fact by some that irregularity in number and distribution contradicts the stomata vera as being anatomical structures. It is true that anatomic structures are generally regular. The irregularity and distribution of the stomata vera may be acquired. Again, Schweigger-Seidel and Dogiel claim that the granular polyhedral cells projecting into the stomata vera are the nuclei of the group of endothelia surrounding the stomata verum. This I have disproved in many specimens, especially those of the embryo of the pig where the nuclei of the endothelial plates surrounding the stomata vera are distinctly shown in the cells themselves, far removed from the edge of the stoma verum. In this work will appear a cut from the lamented Schweigger-Seidel to illustrate his views (1866). One might describe, as Klein does, two kinds of stomata vera, viz.: (a) the stomata vera which connects a lymph channel directly with the peritoneal cavity and (b) a stomata vera which leads into capillary lymphatics from the peritoneal cavity without a channel distinctly lined by granular cells. The most significant variety are those connecting the peritoneum and lymph trunks directly. I mention here a noticeable feature in the lymphatic channels of the diaphragm of a rabbit on the abdominal side. It is that the stomata are far more numerous in the wall of the lymph channel than they are in the peritoneal endothelia immediately over them. The typical stomata vera on the diaphragm are chiefly aggregated over the intertendinous lymph spaces. This is also the chief locality where colored granules collect from the peritoneum when absorbed. It may be that fluid material will pass through the interendothelial space of the peritoneal serosa without a distinct aperture or stoma lined with distinct cells, but that the apertures became more marked and distinct, yes, even lined by granular cells, when they reach the walls of the lymph channels. At least it is very evident in specimens which I have examined that the walls of the lymph channels show stomata much more frequently than the peritoneal endothelia which lie immediately over them. I may be deceived by the closure of the stomata of the peritoneal endothelia. The superior number of stomata in the walls of the lymph channels directly underlying the peritoneal endothelia, which has so few, would indicate more special use. It seems to me that it would indicate that fluid matter could more readily pass through the interendothelial space of the peritoneal serosa than it could through interendothelial space of the lymph wall.

The above is a general sketch of the stomata vera of the peritoneum, but I wish to add some remarks from further experience and also views from literature. Perhaps today there are an equal number of investi-

gators who deny and assert the existence of preformed stomata in the peritoneum. Rather it should be said that the existence of structures known as stomata are recognized, but the interpretation of their structure and function is unsettled.

Tourneaux and Hermann, in the *Journal de l'Anatomie*, twenty years ago made a long research of the peritoneum of the echinoderms, annelides, insects, mollusks, fish, batrachians, reptiles and birds. In the conclusions were noted approximately the following important statements:

1. The endothelia of the serous cavity form a continuous layer without perforations or stomata.
2. That no absolute anatomical distinction exists between epithelium and endothelium; that each verges into the other.

It is the first statement which we have to deal with, and that is the direct denial of the stomata by saying the peritoneal membrane is not interrupted by apertures. However, by more careful observation of the excellent investigations of these French scientists, it appears to be a matter of interpretation, as they acknowledge the existence of what has been called stomata from 1861 to 1896, but they term them a depression in the peritoneum or the wells of Ranvier—merely another name for the same object. According to Ranvier these so-called stomata are lymphatic depressions plugged with leucocytes and the plug is movable. The lymphatic well might appear at any place and as soon as the leucocytes disappear the well would disappear to reappear in some other locality. This, if correct, might account for the irregularity of numbers and distribution.

Ranvier considered the cells which line the stomata as leucocytes. Muscatello asserts in 1896, after a long search, that stomata do not exist and claims that specimens hardened in Muller's fluid and stained in acid fuchsin do not show them. Muscatello tries to explain that at the common junction of several endothelial cells the protoplasmic portion of the cells retract producing the aperture on account of the irritating reagents.

Investigators from the days of Von Recklinghausen (1861), and Ludwig (1866), with his pupils, Schweigger-Seidel, Dybkowsky and Dogiel as well as Klein and Burdon-Sanderson (1872), with Lawdowsky have announced that the peritoneum is not a smooth, non-interrupted membrane, but that here and there small masses of granular cells could be observed. They are situated at the common junction of several endothelial plates and are of protoplasmic character. In the main they are in groups, but often one finds only two surrounding the so called stomavermum. The typical places where they are found are on the abdominal surface of the diaphragm, omenta, frog's mesogaster and frequently on



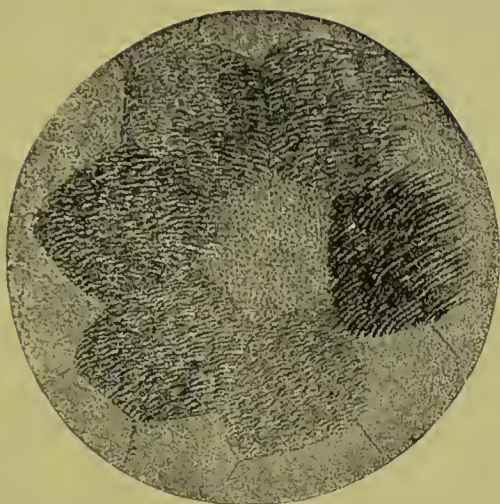


FIG. 55—(A. Kolossow, 1895.) Various developmental phases of the cilia (hair) on the endothelia of the peritoneum of the anterior abdominal wall of a pregnant frog. Kolossow claims that all the peritoneal endothelia possess cilia, or, as he terms it small hairs.

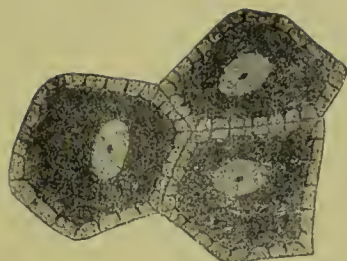


FIG. 57—(A. Kolossow, 1895.) Thin endothelial cells from the pleura-costalis of the cat, bound together by anastomotic processes. The anastomotic processes bind the inferior protoplasmic portion of the cells together while the superior portion the cover-plates have their edges merely in contact. Kolossow claims that the dotted points on the cover-plates correspond to hairs. Specimen treated with osmic acid and tannin.

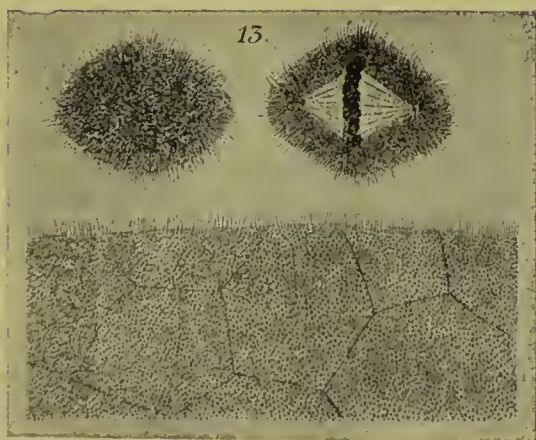


FIG. 56—(A. Kolossow, 1895.) Endothelial covering of the pleural surface of the diaphragm of the rabbit. On the edge appears projections which Dr. Kolossow claims are hairs which are thickly studded over the cover-plate. The upper figures in the cut are from the intestinal peritoneum of a white rat (new-born). The cells are in a state of division (karyokinesis). The cells show spindle shape and short, hair-like projections. (Osmic acid and tannin.)

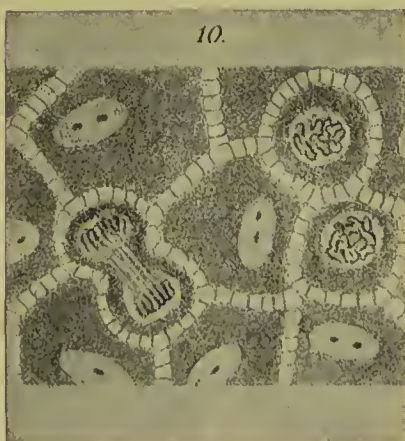


FIG. 58—(A. Kolossow, 1895.) Endothelia from the small intestines of a new-born cat. A, process of cell division is going on, karyokinesis. The interendothelial space shows anastomotic processes binding the cells together.





the mesenteries; especially prominent are these cells on frog's lymph sacs. Klein made the important announcement that these small polyhedral granular cells would multiply in times of inflammation and he termed them germinal endothelia (Keim-endothel). As stated above, Ranvier considered the cells as leucocytes which were caught in the clefts of the endothelia between the lymph sacs and the peritoneum on their journey from one to the other. But it seems to me that the granular cells can be distinguished from leucocytes because the granular, protoplasmic cells have a brilliant ovoid nucleus, but one does not see the brilliant oval nucleus in a leucocyte. Tourneaux and Hermann attribute to the small groups of granular cells the power of reproducing endothelial plates. They are centers of formation, reproduction. With them Ranvier's lymphatic wells are only a depression on the peritoneal surface, and this depression is simply lined by the small growing endothelial plates, which will finally supply the place of the old, wornout and dying endothelia.

These depressions, lymph wells, in the peritoneal surface, similar to a depression in the epithelia of the vagina, do not communicate directly with the subjacent lymph spaces, but according to Tourneaux and Hermann have no relation with any structure except the free surface of the peritoneum. With them they are not channels of communication between the peritoneum and subjacent lymph spaces. It may be remarked that the claim that the stomata connecting the peritoneum with the lymphatics is based on experiments. Those who claim that the stomata directly connect the peritoneum with the lymph channels support it by showing that finely divided matter suspended in fluids, injected into the peritoneum, will rapidly pass in remarkable quantities into the lymphatics of the diaphragm directly beneath the peritoneal surface. This fact I have repeatedly confirmed with carmine but especially Berlin blue in experiments, chiefly on rabbits and dogs. The chief experimenters who claim there is a direct and open communication between the peritoneum and lymph channels by means of stomata are Recklinghausen (1861), Oedmansson (1862), Ludwig (1866), and his pupils, Schweigger-Seidel and Dybkowsky, also Toldt, Klein, Georg Wegner (1877) and several others. Added to this experiment of absorption of solid particles from peritoneal fluid these investigators claim to confirm the existence of the organized channels, stomata, by the use of silver solution which gives to the groups of granular cells a dark red color, a much more intensified color than the endothelial plates. The opponents of the view that these openings are preformed have been such authors as Tourneaux, Hermann, Affannasiew, Pia Foa, Muscatello and Kolossow. These authors claim that the figures produced by the silver solution demonstrating stomata are merely artificial products of the silver, merely precipi-

tates, and the fact of the transportation of the finely divided coloring matter from the peritoneum to the lymph channel of the diaphragm is due to the widening of the interendothelial space allowing the particles to pass, and then the passage closes. I have followed with much care the labors of all the above authors on both sides of the question—stomata or no stomata—and accompanied the study by many experiments of my own. I have learned to admire all the investigators but must, from my own experiments, assume, though it be in opposition to an able galaxy of scientists, that there are preformed openings in the peritoneum.

The peritoneal stomata sketched first, so far as I know, by Oedmansson, of Stockholm, in 1862 and reproduced in this work, are definite objects which cannot be explained away by accidents of reagents nor mere suppositions of anatomic widenings of assumed endothelial cement substances, for I must deny this hypothetic interendothelial cement substance and replace it by the term interendothelial space.

Schweigger-Seidel and Dogiel in 1866 described stomata of a special sort on the cisternae lymphaticae magnae of the frog which they considered produced a direct organized passage from the peritoneal cavity to the lymph cavities below the peritoneum. They then considered that they had a definite proof of Recklinghausen's view of an open, organized communication between the peritoneum and lymph channels. But later authors, as Tourneaux and Hermann, have denied this. In 1895 Muscatello of Turin, Italy, a devoted disciple of Bizzozzero, paid much attention to the idea of preformed peritoneal openings and ultimately denied their existence. Tourneaux and Hermann claimed to have demonstrated that the stomata or what they term depressions are lined with small endothelial cells and that a layer of connective tissue can be seen at the bottom of the depressions which they claim effectually demonstrates that these stomata or depressions are not through and through openings, i. e., they do not form an organized connection between the peritoneum and lymph channels. The peritoneum is with them a closed membrane. Foa, who labored in Recklinghausen's laboratory, and Lawdowsky, both came to the conclusion that the stomata in the lymph sacs of frogs do not pass entirely through the wall of the lymph sac, but merely form depressions on each side. From the time Oedmansson first drew cuts of the stomata spuria and vera, investigators have been attempting to interpret their meaning. It would appear that the main modern tendency is to interpret the so-called stomata as centers of reproduction of endothelial plates to supply the place of dying comrades. Experiments of Von Recklinghausen, 1861, first showed that the abdominal serosa of the diaphragm was the only or chief point of absorption for injected matter into the peritoneum. In 1882 Dubar and Remy showed the presence of points of absorption outside of



the diaphragm. They showed that absorption of injected material not only occurred along the lymph channels of the diaphragm but along the blood channels, that is, the portal vein and its branches. In 1882 Maffucci made extensive experimental investigations and announced that almost the entire visceral supports would absorb, as the omenta, ligamentum latum, Douglas fold, mesorectum and mesenterium.

This generalized the peritoneal surface as an absorbent for injected material. But modern experiments tend to the view that the diaphragm is the chief and main point of absorption of injected material, which has been well demonstrated by Muscatello, in 1895. Yet after many experiments with carmine or Berlin blue suspended in the injected fluid, especially in dogs and rabbits, I am convinced that the colored granules become not only extensively deposited in the lymphatics of the diaphragm but also in the lymphatics of the gastro-splenic omentum. The conviction comes from careful microscopic test after the animal is killed.

Kolossow states in his investigations that he has been able to dis-

FIG. 59—(Author.) Young dog's gastro-hepatic omentum. (Oc. 4, ob. 3, R.) A group of 10 cells surrounding a stoma verum with two nuclei. 1, nucleus of stoma verum; 2, stomata vera cell or guard cell; 3, one of the group of endothelia; 5, rift between endothelial plates.



tinguish the organic anastomotic connection of the germinal endothelia surrounding the stomata vera, or what he terms merely centers of reproduction of endothelial plates. This is not a strange assertion, for from my investigations I am now thoroughly convinced that the endothelia of the blood vascular system and of the lymph vascular system and the peritoneal endothelia are each and every one organically bound together. The endothelia of each one of the three systems are similarly connected by anastomotic processes. These processes chiefly and essentially bind together the underlying protoplasmic portion of the endothelial cells and allow more or less freedom of the cover-plate; i. e., the free surface or indurated portion of the endothelial plate is not bound together with as many protoplasmic processes, at least, as the protoplasmic portion of the cell. The protoplasmic connection of the germinal endothelia surrounding the stomata vera furnishes no especial argument against the existence of preformed or primordial openings. Kolossow asserts that he has been able to find cilia on the germinal endothelia surrounding the stomata. However, so far in my investigations I have not been thoroughly convinced that I can confirm his discoveries. We have

examined many animals and noted the germinal endothelia surrounding the stomata vera and have noted that the surface of germinal cell is not especially even or smooth, that it is not uniformly colored, having lighter and darker shades and spots, and that there appear very small projections or spiculi on the cells, but we had not considered them sufficiently prominent to deserve the name of cilia. The spots on the germinal endothelia surrounding the stomata vera which are quite dark red are probably projections of fresh young protoplasm, containing considerable perceptible albumen, or perhaps these projections are covered with a thin capillary layer of blood plasma or lymph which endows them with the power of precipitating Ag. NO<sub>3</sub>.

In regard to the stomata spuria, there is little to add to what has been said in respect to the endothelial space. On the interendothelial line there exists a peculiar dark spot after the application of silver nitrate. This dark spot is termed stoma spurium. It was thought by Virchow to be a lymphoid cell. It was called by Oedmansson, Von Recklinghausen and Klein a connective tissue corpuscle projecting from below upward between the endothelial plates, or rather, projecting upward through the interendothelial space. It is perhaps a source of new endothelia. It may be a leucocyte caught on the way while journeying from the subperitoneal spaces to the peritoneal cavity. I am not fully satisfied as to the many appearances on the interendothelial line which are ascribed to what is known as stoma spurium. Doubtless it is correct in the vast majority of instances, when one examines a non-traumatized, or better, a normal peritoneal membrane carefully stained with Ag. NO<sub>3</sub> to consider the peculiar round or oval dark spot on the interendothelial line as a stoma spurium. For we must acknowledge that if a rabbit is killed without trauma to the peritoneum, and the peritoneum stained and hardened in situ, that such a peritoneum is normal to that animal. Besides, when we find this same phenomenon on every mammal examined, we must also acknowledge that such an appearance is a normal phenomenon of animal life. As a matter of fact, the dark spot appears on the interendothelial line in the peritoneum of all animals stained by Ag. NO<sub>3</sub>. Hence we must consider it an anatomical structure. It is true that its number and distribution is absolutely irregular. But it does not explain away the fact of existence of the stoma spurium to say that it is an artificial produce of the silver solution and also that it is incident to trauma because trauma increases the appearance of spots similar to it. Neither can it be considered of a traumatic origin when it is found in all mammals examined under all precautions. Hence, at this stage of knowledge of the peritoneum, it is recognized by nearly all investigators that the structure known as stoma spurium situated on the interendothelial line is a normal structure, and

that the majority interpret it as a connective tissue corpuscle projecting upward between the endothelial cells.

The shape of the endothelia varies in different animals, and is different in the embryo and adult. However, generally analogous shaped endothelia are found in analogous localities. Affannasiew, Muscatello and others appear to think that the general and original form of an endothe-



FIG. 60—(Author.) Drawn from rabbit's mesentery. It was pencilled in situ (brushed four to six times);  $\frac{1}{2}$  per cent. solution  $\text{Ag. NO}_3$  was then poured over it for two or three minutes and preserved in formaline two parts and water one part. The specimen was mounted in glycerine, and drawn as close as possible to Oe. 2, ob. 8a, Reichert. 1, endothelium; 2, 2, rift between the endothelia with precipitated deposit; 3, 3, 3, 8, stomata vera; 4, stomata spuria; 5 and 6, nuclei; 7, points to the interendothelial stomata; 9, closed stomata vera; 8, with the germinal cells dropped out. The endothelium containing the nucleus. 6, is the center of a group of 5 cells. The central cell is doubtless the germinating cell, which corresponds to a stoma verum. This specimen is from a tract or cord of germinating endothelium. The surfaces of the endothelia are quite rough after being pencilled with cotton on a toothpick and  $\text{Ag. NO}_3$  applied, open and closed dots appear along the interendothelial spaces. Perhaps the open dots or rings represent retractions of interendothelial matter. The black dots represent precipitates from the  $\text{Ag. NO}_3$  still in the clear open rings along the interendothelial lines, the granular polyhedral cells may have dropped out. The dots and rings were drawn only on endothelia No. 7.

lial cell is polygonal. My examinations include the horse, cat, pig, woodpecker, shy-poke, hen, dove, gopher, turtle, toad, dog, rabbit, frog and the embryos of pig and man. So far in the examinations I cannot generalize any single form of endothelial cell. It is probable that the polygonal form outnumbered any other single form. Again, the endothelial cells or plates are quite different in the embryo than the adult.



The frog possesses the largest and most irregular forms of endothelia of any animal examined. In the horse and cat are found large areas of uniformly shaped endothelia.

The outlines of the peritoneal endothelia are generally those of curves and straight lines. Occasionally we find curved and straight outlines. But sinuous outlines is the chief characteristic of endothelia covering lymph channels. Some endothelia are perfectly round, some show obtuse angles, while others show acute angles. They may be oval, square, spindle-shaped or present graceful loops and necks or assume the shape of a rectangle. They may be triangular (pig), pentagonal or hexagonal. The length may exceed the diameter, perhaps by six times. But the peritoneal endothelia does not vary in size, like the endothelia covering lymph channels, in many cases, especially in embryos or new-born.

The long, rectangular shaped endothelial plate may assume a bent or curved shape to accommodate blood vessels and trabeculae. A peculiar appearance is lent to the surface of the endothelium by the reception of an acutely pointed angle in the recess of two or more other endothelia. The uniform shape of considerable areas of endothelial surface in some animals produces a beautiful mosaic, which is occasionally only varied by stomata vera and spuria. In other regions the delicate mosaic is relieved of its uniformity, especially in the omenta, by germination and vacuolation of cells, while streaks of fat globules may come in to vary the scene.

The varied shape of the endothelia found in the adult body I am now fully convinced is an acquired condition and that the early embryo possesses a much greater uniformity than the adult. The acquired shape of the endothelia of adult life rests on two factors, viz.: (a) the elastic character of the endothelial plate and (b) the expansion and contraction of vessels (blood and lymph) and organs. Another factor may arise which I have so far been unable to settle, and that is in the regeneration of endothelia there may be different sized and hence different shaped endothelia formed. But perhaps this hypothesis may rest on the idea that endothelial plates are originally of the same size, but that one plate is subject to expansion while the other is subject to contraction, and the plate is so incidentally found in one or the other condition. So far as the elastic feature of endothelial plates is concerned, it is a well-known clinical fact. The distension accompanying ascites and the sudden contraction of the peritoneal endothelia immediately following the evacuation of the ascitic fluid is sufficient proof. Of course, the chief elastic force lies in the subserous elastic fibre. But the endothelial plate must contract very much to readapt itself to its original form. If it did not contract enormously the edges would overlap. However,

we must not allot too much elasticity to the endothelial plate, for the interendothelial meshwork also no doubt shared in the expansion and actually shared in the contraction. This view sounds the more reasonable when in all probability ascitic fluid rests on peritoneal inflammatory origin, hence, the very œdema alone accompanying peritonitis would expand the interendothelial substance. In experiments I note that alcohol and formaline contract the endothelia while water expands them. Again, by observing many specimens occasionally one will arise where the bent or curved endothelium will spring forward and backward in the waving water, such condition being best observed by elevating the cover glass above the slide sufficiently to allow free fluid currents in various directions. On organs where the endothelia are quite fixed, but which expand and contract in a rhythm, the endothelial plate no doubt expands and contracts, yet some of their adjustability must be due to the interendothelial space.

The acquired shape of the endothelial plates from expansion and contraction of vessels due to emptying of fluid I have investigated considerably. In one of the figures is sketched a typical sample in a frog's

FIG. 61—(Author.) Endothelia of crawfish of about 2½ inches long. 1, 2, 3, stomata vera; 4, 5, stomata spuria; 6, 7, 8, nuclei; 9, 10, endothelia. Ag.  $\text{NO}_3$ , ½ per cent. applied. The endothelia of the crawfish are relatively small. The stomata vera are plain. Under the endothelia in many places are vast beds of lymphatics. This specimen was taken from the intestinal tract. The interendothelial line is quite small and is not so easily divided into two parallel lines with transverse anastomosing processes. This crawfish was secured in Lake Emily, a fresh water lake of Wisconsin.



mesentery where the endothelia covering the blood vessels are enormously elongated transversely over the vessel from its emptying and filling. Of course, an elastic endothelial plate is like rubber which, being repeatedly stretched, loses some of its original shape and gradually assumes a shape in accord with the direction of its chief force. Now the acquired shape of endothelia is not alone due to the emptying and filling of blood and lymph vessels, but their shape may be gradually moulded by fixed organs which have a definite movement to go through; for example, the stomach is fixed at the pylorus and the entrance of the œsophagus through the diaphragm, but the chief portion of the stomach as it empties and fills repeatedly passes, no doubt, through the same motions, and in this manner certain portions of the peritoneal endothelia will acquire a shape peculiar to the direction of the chief force. The same may be said of the expanding and contracting bladder. Whatever the factors be, the endothelia assume a wonderfully varied shape, a multiform outline; in other words, the major and minor diameters vary in a wide degree. It may be noticed that on the mesentery and diaphragm of the horse, cat, dog, and other animals, organs which pos-

sess a high range of motion, the endothelia are smaller and more uniform than on many other places. In adult animals the endothelia are subject to a wide range of shape. The shape of the endothelia in embryos and new-born also has a wide range. I have noticed in the embryo pig enormous numbers of triangular endothelia and also those having the shape of a cone with a curve for a base. Another element which produces acquired changes in the shape of endothelia of both new-born and adults is the development and disappearance of fat. Fat globules simply collect and expand in a connective tissue corpuscle, and as the fat globules accumulate and expand the endothelia immediately over them acquire new shapes. The endothelia on the top or summit of the fat globule became much more varied in shape than the endothelia at the base circumference of the fat globules. I sketched several figures in fields of fat globules to show the very marked variation in the shape of the endothelia covering them. Under histology should be included the shape of the germinating and vacuolating cell, for these are simply renewing the place of dying comrades. It may be stated in general that the shape of germinating or growing cells includes all shapes from polyhedral to polygonal. The granular polyhedral cells lining the vertical lymph channels, or composing the stomata vera, is one typical set. The innumerable round and oval form accompanying many tracts of germination represent another set.

The arrangement of the endothelia is a subject of more significance than its shape, for in the arrangement appears to be the original physiologic indication. It appears to me that the pig embryo shows more definite arrangement in its endothelia than any of the above mentioned animals. One can note very symmetrical arrangements of endothelial plates around stomata vera, on the gastro-splenic omentum, and quite symmetrical. In other portions of the pig's peritoneum the triangular shape of some endothelial plates allows a symmetrical mosaic to be produced. The first arrangement of endothelia to which attention may be called is that around stomata vera. The stomata vera are situated at the common junction of 3 to 14 endothelial plates. In the embryo pig there exists the most typical and symmetrical arrangement of endothelia about the stomata. The endothelia assume a cone-shape and their sharp points meet in common about the stomata. It would appear that in these embryos the symmetrical arrangement of endothelia about stomata vera was a design of nature to accomplish the purpose of physiology in the peritoneum. I am convinced they are preformed openings, original, anatomic and physiologic structures for the purpose of holding in definite relation the peritoneal cavity and the sub-peritoneal lymph channels. The circumferential edges of the stomata vera are lined with granular, polyhedral, young cells, around which are



symmetrically or otherwise placed endothelia, the stomata vera and the common junction of endothelial plates.

The endothelia tend to group themselves about stomata vera. The number of endothelia composing the group include from three to fourteen cell plates. The best samples of endothelia around stomata I found in the embryo pig, but the frog, turtle, and other animals also produce good samples.

Again, there is a tendency for the endothelium, especially in early embryos and even adults, to arrange themselves in relation to blood vessels. The elongated shape of the plate is easy to make out grouped in large numbers along the course of the blood vessels. Great whirls of long rectangular endothelia, curved to suit the course of the vessel as it lies in a trabecula, may be plainly viewed in embryos and to a less typical condition in adults.

If one examines the centrum tendineum of the diaphragmatica peri-

FIG. 62—(Author.) Two endothelial groups taken from two places of a horse's mesentery. 1, 1, point to the stomata vera in A and B. 2 in A is a nucleus; 2, 2, in B points to 5 apertures which appear like stomata vera; 4, 4, in A and B, are endothelia; 5, 5, stomata vera; 6, a black point on endothelial cell. In B it appears that the stomata vera are peeking up through the endothelia, or they are shimmering through.



toneum under the microscope, long, straight, dark and light streaks may be observed. The dark streaks, or cords, are the tendons of the diaphragm, while the light streaks are the spaces between the tendons. By careful observations of the abdominal diaphragmatic serosa it will become apparent that the endothelia covering the tendinous or dark streaks is of a larger size than that covering the light spaces. Hence, the arrangement of the endothelia occur in two different tracts over the diaphragm. The explanation first given to the phenomenon by Ludwig and Schweigger-Seidel was that the endothelia covering the light spaces between the tendon bundles of the diaphragm was over lymph channels. In other words, the lighter inter-tendinous spaces are really long lymph tracts, and as endothelia approach lymph channels it is known to change its outlines and become more sinuous, irregular and smaller. For the purpose of demonstrating the arrangement of the endothelia on the tendinous and inter-tendinous portions of the diaphragmatic abdominal serosa, the rabbit is first recommended; however,

the dog and guinea-pig we found equally good. So far as the arrangement of the peritoneal endothelia is concerned, it is simply irregular. The irregularly shaped endothelia become arranged so that they wrap themselves around the trabecula, leaving no subserous tissue exposed to the abdominal cavity. The endothelia were generally found more irregular in shape and arrangement on the viscera than on the omenta, diaphragm and parietes. No doubt this greater irregularity of shape and arrangement on the viscera is due to the greater and wider motion of viscera. The shape and arrangement of endothelia differs in different organs and even in the same organs of the same animal. The wide variation in shape and arrangement of peritoneal endothelia must rest in (a) original or (b) acquired condition. What the primordial arrangement of the endothelia is we are not yet informed, though some think the original shape of the endothelia are polygonal and others think they are preformed about stomata vera. From my own work it appears there is ground for primordial arrangement of endothelia, and it appears to me that they are preformed about stomata vera.

As to shape of endothelia, it appears probable that they were originally polygonal. The acquired shape and arrangement of endothelia is a matter which rests more on physical forces of a tangible nature; motion, friction, expansion and contraction unfold a long evolutionary story in required conditions of the endothelia of the free surface of the peritoneum.

The regeneration of peritoneal endothelia will be considered here only as it belongs to the normal peritoneum. It does not partake of the domain of pathology. The regeneration of peritoneal endothelia will simply be considered as a natural physiologic process whereby new endothelia are produced to supply the ranks of dying or worn-out comrades. The endothelia of the peritoneum are like a standing army which demands a steady recruiting to supply the various kinds of loss. The peritoneum being an organ of intense vascular and functional activity its needs are vast, which are fully supplied by what we shall term germinating endothelia. We might call these young or germinating endothelia, but I adopt the term germinating from Klein as an apt expression. One soon has the attention drawn to germinating endothelia in the microscopical study of the peritoneum. If an omentum majus or gastro-splenic, fresh from man, rabbit, dog or any animal so far examined be carefully placed in a solution of 1-8 per cent to 1-2 per cent of Ag. NO<sub>3</sub> and the sunlight carefully regulated with distilled water, one will soon be able to detect with the naked eye brown spots or patches, and when these brown patches are mounted in glycerine under the microscope they are seen to be chiefly composed of germinating endothelia. The young growing protoplasmic cell readily

stains a dark brown with the silver solution. Germinating endothelia present all grades of cells from the polyhedral to the multiple-sided, sinuous bordered cells. These germinating endothelia are apt to be found along large blood vessels.

We may first note that germinal endothelia grow over the surface of the common endothelia so much that it requires a different focus. They grow in heaps or cones or irregular elevations. The growth may resemble a network. The silver salts stain the growing cells very brown. The germinating endothelia are of all grades, sizes and shapes. The

FIG. 63 —(Author.) Drawn from the mesogaster of a mud-turtle near the oesophagus. The turtle weighed about two lbs. The specimen represents two stomata vera with ten granular polyhedral, nucleated cells surrounding it and two stomata vera surrounded with 7, 3 and 4, nuclei of the common endothelia 5 and 6. Ag.  $\text{NO}_3$  applied  $\frac{1}{2}$  per cent. strength. A turtle has no omentum. These stomata resemble the stomata on the lymph sacs of the turtle. This specimen was stained in situ while the animal's heart still beat (as it will for hours after decapitation) and all trauma avoided as much as possible. The turtle presents the most beautiful and perfect outlined nucleus of all the animals. Its nucleus is characterized by its size, about  $\frac{1}{3}$  of the size of the endothelium, by its almost exactly round outline being often slightly elongated in one axis. It possesses one or more distinct nucleoli and it is more centrally located than it is in most animals. In the breeding season (July) it is very plain on the peritoneum covering the ova sac when rapid germination goes on. A vast system of pigment connective tissue corpuscles are then seen under the peritoneum of the ova sac. Also at this time may be observed many ciliated endothelial cells as is found in the frog during gestation—perhaps due to friction and high blood supply. 7, 8, 9, represent the interendothelial space with its two parallel lines and transverse anastomosing processes (which I have likened to a railroad track).



fields of a germinating endothelia are found well on the omenta of dogs and rabbits. But the most typical and beautiful cases of germinal endothelial growths I found on the frog. Doubtless the typical cases were from frogs with local peritonitis, as quite a number of times I have studied the growths, but not more than a quarter of the cases would show the typical germinal endothelial growth. The mesentery of a frog often shows beautiful cord-like growths which radiate like a network and, meeting other cords or trabecula, anastomose. But we may in general expect to find a rich growth of germinal endothelia on the omenta of dogs and rabbits which colors intensely with Ag.  $\text{NO}_3$ . We may find isolated nodules or cords well elevated above the common endothelial surface, distinctly defined in outline and shape. Some of the nodules have a well-defined stalk and large bulb at the end so that the whole nodule resembles a base-ball bat. The intense brown color which the germinating endothelia assumes upon the application of Ag.  $\text{NO}_3$  characterizes the cells as young and growing protoplasmic cells with considerable perceptible albumen. They grow in irregular tracts, cords or nodules and especially common from the edges of the lymph spaces and



vacuolated cells. In general it may be asserted that stomata vera are a chief source of the germinating cells, and the stoma spurium a second source. It is difficult to avoid the general impression that there are definite centers around which the germinal cells grow and cluster. For a long time I studied the germinating endothelia for the purpose of classifying it, but have never come to any satisfactory conclusion. But we may designate three classes for convenience: (a) nodules, (b) cords and (c) patches. It may be that cords and patches may result from the fusion of nodules. I have found much difficulty in obtaining access to any extensive literature in regard to germinating endothelia. All that is at command at the present writing are a few short paragraphs by the excellent histologist, E. Klein, of London, England.

In the first place it may be noted that germinating endothelium of normal peritoneum differs from a pathologic process but little, that it may be very difficult, even for an expert, to make out the difference between germinating endothelium, i. e., regenerating endothelium and the proliferating endothelium of an inflammatory process. In some cases it seems impossible to decide when studying the omentum of animals whether the process is actual regenerating germinal endothelium or the result of a chronic peritonitis. These remarks are entirely confined to normal and regenerating germinal endothelium. Neither do I wish to enter upon the subject of the subperitoneal lymph vessels only so far as to discuss the very significant vacuolated cells—a method of proliferating or multiplying endothelial plates.

Active germinating endothelia may be found on the omentum of man, frog, dog, and rabbit, on some of which it is rich and abundant. Splendid specimens of germinating endothelium may be found on the lateral portions of rather wide trabeculae. If one places a portion of a frog's mesentery under the microscope mounted in glycerine the typical germinal growths may be observed. First may be noted nodules or club-shaped bunches of endothelia projecting above the common surface endothelia. The nodule or club has a small constricted neck or stalk which starts distinctly from the common surface. Some have the shape of a half sphere, and the flat surface rests on the endothelia. The stalk appears to me to have its origin from (a) stomata vera, (b) stomata spuria, (c) from the circumference of a lymph sinus. The lymph sinus may be a vacuolated cell. It seems that the endothelial cells have grown in such a shape as to fit the nodule. Sometimes the nodule resembles a cone. The stalk may be composed of one or several endothelial cells. Two nodules may originate from the same point, and this view induces me to consider both stalks as originating from a stoma verum. In the same specimens may be observed cords of germi-

nating endothelia which run in various directions. But they lie chiefly on the circumference of lymph sinuses.

No doubt such areas of germinating endothelium is what Klein designated as peri-lymphangial. The vacuolating cells seem to multiply the endothelium indefinitely and thus form new lymph channels and sinuses. The endothelial cells grow from the circumference of the areas of vacuolated cells and are very variable in extent. The cells may be single, in rows or united into large patches. The new growing cells may have one, two or three nuclei.

The patches or tracts of germinating cells are only the result of fusion of the cords. The blood vessels of these patches are not easy to make out in their relations and development. Neither have I fully satisfied myself in regard to the nerve supply in its chief relations.

A feature in regard to the germinal endothelia is that they are not so easily brushed away as the normal. The normal endothelium is very

FIG. 64—This beautiful and natural cut of the endothelium of the diaphragm is by Dr. Piersol. It is stained with silver nitrate; *n* shows the nucleus of the endothelial cell; *s* points to an intercellular cleft, or to what is generally known as a pseudo-stoma. The silver salt precipitates the albuminoid substance between the endothelial cells, and demonstrates accurately their outlines. Fluid substances can penetrate between the endothelial cells and also the pseudo-stomata.



easily broken away from its attachments. For example, from the slight trauma caused by carrying portions of the peritoneum of cows and sheep from the fresh slaughter-house to the laboratory, the normal endothelium is badly desquamated, but the germinating endothelia is so much more adherent that it is more easily and accurately studied.

The omentum of the dog and rabbit may be literally covered with buds of growing endothelia. There is a difference in not only the age of the same species of animal, but also a difference in the same animals at the same age. It would appear that germinating cells multiply with considerable rapidity as one can notice nuclei in one cell indicating a division but also may be lymphoid corpuscles, which are both attached and detached. Lymphoid cells, two nucleated cells, as well as many small varied shaped cells in abundant numbers in the microscopic field indicate rapid multiplication. It is well to study the pregnant frog or

turtle, for whenever the large ova sac frictionizes against the peritoneum there is apt to arise ciliated endothelial cells but also germinating cords or tracts. The ciliated cells are found in rows and circle around the stomata vera, on the pedicle of the widely distended ova sac. The cilia stain intensely with Ag. NO<sub>3</sub> and these ciliated cells are of a germinating character, in fact, some definitely surround stomata. Not infrequently may be observed two club-shaped nodules springing from a stoma verum; merely the points at the bottom of the stalk come in contact. During many months of microscopical investigations it was very apparent that germinal endothelia occupies a close relation to the lymph channels and cell vacuolation, as well as being distributed along blood vessels. The lymph channels are a result of vacuolation, i. e., a multiplication of endothelial cells; one can observe all the grades of formation from the smallest point to large lymph sinuses. The lymph channels, or, better, the sinuses, gradually broaden and the multiplication of cells occurs from the circumference. The outlines of the cells become more pronounced in their sinuosity as the lymph sinus increases in size. Very frequently, however, the outlines of the endothelia multiplying to form the lymph channel preserve quite a straight outline until the vacuolation has half a dozen endothelia to cover in its space, after which time the endothelia covering the dome of the vacuolation assume sinuous borders, and soon after the sinuous bordered endothelia arise stomata are apt to be noticed at the bottom of the vacuolated lymph sinus. Vacuolation is a method by which endothelial cells multiply and enclose a space, as a lymph channel or lymph sinus. The endothelial cells covering the vacuolation are of all sizes and shapes. But the cells covering the highest point of the vacuolation are generally large and appear stretched, as if covering a space filled with distending material—fluid lymph.

Klein mentions that he was enabled by the distance which germinating endothelia stand apart from each other to notice connective tissue corpuscles between the edges of the germinating endothelia. It is easy to observe gross nuclear divisions in the endothelia of germinating character, i. e., one sees two nuclei touching each other in the same endothelial plate, as if they had just divided; other nuclei may be seen with constrictions in them as if they were about to divide, but to observe all the processes, or any definite stages of the well-known karyokinesis, is not so easy. But the subject of karyokinesis is so well established that it is superfluous to insist that peritoneal endothelia multiply by the process of karyokinesis exactly as other cell multiplication.

But Ranvier claims that the lymphatic wells never open directly in the lymph vessels immediately beneath. The claim that first washing the serosa with distilled water will lessen the appearances of the interen-



dothelial structure is not true so far as my experience is concerned. It may be claimed that the interendothelial structures have no reference to the absorption of solid particles held in suspension in peritoneal fluids, because these structures are found where we can find no solid particles absorbed.

The peculiarly typical stomata situated on the amphibian lymph sacs which Schweigger-Seidel, Dogiel and Dybkowsky under the supervision of Ludwig considered as through and through apertures, i. e., direct communication between the peritoneum and lymph sacs, Tourneaux and Hermann claim are not direct open communications between peritoneum and lymph sacs, but that such stomata are the same as the lymphatic wells of Ranvier, i. e., mere depressions in the peritoneal

FIG. 65—(Author.) Omentum majus of sheep. (Oc. 4, ob. 8a., R.). The sheep presents plain stomata vera, but they lack, at least in some specimens, the symmetrical arrangement of the polyhedral granular cells which exist on the septum cisterna lymphatic magna of the frog. Besides the cells of the stomata vera 1, 2, 3, 4, 5, point to typical stomata vera. Many of the germinal cells in the stomata vera are multi-nucleated.



surfaces. The floor of the well is lined with small endothelial cells. Foa claimed that no aperture occurs in endothelia of lymph sacs, but that the apertures are situated in the ground substance. I believe that my researches, specimens and cuts will absolutely disprove Foa's assertion. Ranvier goes so far as to say stomata are only apertures after the leucocytes have passed out. Some of the apertures are difficult to interpret. There are various kinds of rings and ovals. Some rings have a small circumference and a large clear space, others have a large, wide black ring with a small, clear space, or the whole fleck may be black disc. It is observed by all investigators that trauma increases the number and size of the interendothelial rings and discs, so does distension of tissue or inflammation. This is easily studied by stripping off small pieces of peritoneum after it has been stretched over a distended stomach or bowel. If this endothelial membrane be stretched, we do not see after 1-4 per cent Ag. NO<sub>3</sub> solution be applied the narrow uniform linear cleft between the endothelia, but the interen-

dothelial cleft appears like a rosary. The parallel lines are disturbed in their uniform relation to each other and in their place may be observed rings, ovals, black discs and irregular spots with or without clear spaces in the center. These interendothelial objects are seldom uniform. The irregular objects which appear on the interendothelial space after the application of Ag. NO<sub>3</sub> and stretching are doubtless due to the irregular breaking or thinning of the anastomotic protoplasmic processes and not merely to the hypothetic, semi-fluid, cement substance. It may be stated that by the careful use of osmic acid and reduction by tannin the outline of the nucleus in the cover-plate may be seen, although the nucleus does not belong to the cover-plate but to the subjacent portion of the endothelial cell protoplasm.

If a microscopic section be made parallel to the interendothelial space the interendothelial tunnels or canals may be observed, which will be illustrated by an excellent figure borrowed from Dr. Kolossow, No.

page . At the common junction of several cells where the interendothelial anastomotic processes are less numerous but longer, the canals are wider. The interendothelial anastomotic processes do not always pass directly transverse from one endothelial cell to another, but may be oblique or even sinuous and they may branch so that circular openings may appear as the silver is applied. For the typical observation of the endothelial anastomotic protoplasmic processes, I recommend the frog's and turtle's peritoneum fixed by osmic acid, silvered and reduced by tannin. Pieces of peritoneum from the dilating and contracting stomach show well. The anastomosis between the granular polyhedral cells lining the stomata vera, both on the peritoneum and lymph sacs of amphibians and the adjacent common endothelial plates, do not present such marked protoplasmic processes as in common endothelia, but presents more of a protoplasmic network less distinct in stained ones. Kolossow denies that the localities of the small cells (i. e., the stomata vera, the endothelial depression or lymphatic wells of Ranvier) are places for reproduction of endothelia, both to supply the ranks of the dying and worn-out comrades and also to cover the increasing surface of the peritoneum in growing animals. It is true that by carefully preparing (especially of the frog's and turtle's) specimens with osmic acid and tannin one can note the varied changes in the nucleus, especially in the region of the pregnant ova sac. And there are reasons to believe that the endothelial plates increase by karyokinesis. The endothelia as they gradually increase do not entirely lose their organic protoplasmic connection, but the nucleus so divides and constricts that when an additional endothelium is produced it is adjusted by anastomotic, protoplasmic processes to its final adult position. In fresh preparations the nucleus is wonderfully active, but particularly so

in the peritoneum covering the gestating ova sac of frogs and turtles and in localities of peritonitis. We must view the free peritoneal endothelial surface as consisting of large endothelial plates and small protoplasmic cells.

Deckhuyzen held that certain structural changes of the nucleus indicated that the endothelial cell was dying. Kolosow denies that the small protoplasmic cells are centers of formation or regeneration and attributes the reproduction of cells to karyokinesis. If the small protoplasmic cells, which Ranvier calls leucocytes, possess cilia as Kolosow claims, they of course cannot be leucocytes.

In regard to anastomosis of endothelial cells, Lawdowsky said it was impossible and Subbotin said that the endothelia were held together by invisible processes, at least in the vascular endothelia. With silver solutions, osmic acid and tannin reduction, perhaps these investigators would not now assert the above. Of course, the endothelia are extremely thin in the blood vascular and lymph vascular system. However, the endothelia in the large arteries and veins are quite thick.



FIG. 66—(Author.) Young dog's gastrosplenic omentum. Endothelia of the peritoneum fallen off by rubbing and handling what is seen here on lymph endothelia which covers lymph spaces and vessels. (Oc. 2, ob. 8a.) 3, 3, stomata vera; 1, 1, 1, stomata spuria; 2, 2, shows a rift in the germinating protoplasm and has shrunk away from the parts or it has fallen out. 4, 4, 4, nuclei; 5, 5, 5, endothelia; 6, 6, 6, new germinating endothelia around stomata vera 3. 5, common endothelia. 3, open stomata spuria.

A point of considerable interest is in the idea that the cover-plate itself is not elastic. If it be not elastic, then when the linear cleft be once separated it will not return to its original position, and it may be that many of the peculiar figures created by the application of silver salts depend on this non-elastic property of the cover-plate. The protoplasmic portion of the cell, i. e., the portion under the cover-plate, is very changeable and does not lie in compact masses like cells of a beehive comb. The endothelial cells may not fill the entire space, but are held together by the anastomotic processes. As the cells swell the anastomotic processes shorten and thicken, and as the cells contract the anastomotic process becomes longer and thinner. One can at once



observe what vast and active processes could proceed in the interendothelial space. It should also be noted that the peritoneum not only has a membrana limitans, but that such a membrane exists immediately under the vascular endothelia and that it is perforated at points convenient for fluids to pass through the linear interendothelial space and then through the perforations in the adjacent membrana limitans.

Preformed openings are denied by Kolossow and Muscatello. In the place of preformed openings in the blood vascular and lymph vascular systems and free peritoneal endothelia are substituted temporary openings. That is, openings to meet the necessity of fluid pressure. Under raised pressure the leucocytes would find their way through the linear interendothelial cleft or space from the blood vascular, lymph vascular system and free peritoneal endothelia. The above authors seem to believe that the temporary openings are physiological, that under normal conditions the leucocytes form a temporary opening as they require, and under abnormal condition the temporary openings are rapidly increased in number.

#### CONCLUSIONS IN REGARD TO PERITONEAL ENDOTHELIA.

1. The endothelium is the essential structure for physiologic and mechanical function of the peritoneum.

2. The peritoneal endothelium line an enormous lymph sac about equal in area to the skin which originated from fluid pressure and independent motion of viscera and body wall.

3. There are four distinct elements in the free peritoneal serosa, viz., (a) the endothelial plate, (b) the stoma verum, (c) the stoma spurium, (d) the interendothelial space.

4. The endothelial cell is composed of (a) a cover-plate, i. e., an indurated metamorphized protoplasm which is not connected to its fellow by anastomotic processes, but simply rests against its fellow by its edge; (b) of a mass of protoplasm containing a nucleus. This portion of the cell is connected to the adjacent ones by anastomotic protoplasmic processes. The endothelial cell is elastic, and perhaps by its contractility and expansibility it controls to a great extent the interendothelial space. Some endothelial cells possess cilia, but I do not think that the finding of cilia is sufficient to change the name of the endothelial cell to epithelial. The endothelium is an elastic connective tissue corpuscle flattened and smooth on one side and oval or irregular on the other side with various anastomotic processes jutting from it. It contains a sharply defined nucleus, centrally or excentrically located. The plate is covered by an albuminous, semi-fluid substance, precipitated by  $\text{Ag. NO}_3$ , and is probably originally polygonal in shape. However, the plate acquires a very varied shape from living forces. It contains a reticulated network. There are certain apertures in the plate which I

have designated intraendothelial stomata, but later study may indicate that the light spots seen may be perforations in the membrana limitans, but so far have not been able to define their structure or function. It may be that such interendothelial stomata are the result of trauma or reagents. The utility of the peritoneal plate is maximum motion with minimum friction, also it localizes and fixes interendothelial structures, i. e., it adjusts the interendothelial structures, stomata vera, vertical canals to regulate fluid currents and grow endothelia with stomata spuria which are additional points where endothelia can renew itself.

5. Stomata vera are vertical canals located at the common junction of several endothelial plates lined by germinal, granular, polyhedral nucleated cells. The canal opens with one end in the peritoneal cavity and the other end into the subperitoneal lymph channels. A second kind of stomata vera are those which represent simply a discontinuity between the peritoneal endothelia with no mouth lined by granular cells, but granular polyhedral cells. These stomata vera or vertical canals regulate fluid currents and are the source of new endothelia. The septa cisternae lymphaticae magnae of frog show typical examples. It may be that rapid death from perforative peritonitis is caused by the stomata vera quickly absorbing toxic microbes. The stomata vera appear to possess an elastic sphincter to control the degree of opening or closing of the mouth. The granular cells of the stomata vera on the application of  $\text{Ag. NO}_3$  become dark brown or reddish, which doubtless indicates that they are young and contain more precipitable albumen than the adjacent endothelia.

6. The stomata spuria are located on an interendothelial line. The application of  $\text{Ag. NO}_3$  produces a black dot or droplet-like appearance. The stomata spuria are likely connective tissue corpuscles or their processes projecting upward between the endothelial plates. They have been compared to lymphoid corpuscles. They are not likely accumulated products or reagents or trauma. They are no doubt also sources of new endothelia.

7. The chief arguments against the existence of stomata vera and spuria in the peritoneal serosa are their irregular distribution and number, and also that they are accidental products of trauma or reagents.

8. The interendothelial space is no more hypothetical cement substance but a space consisting of a network of interlacing anastomotic protoplasmic processes. The reason there is a network of dark interendothelial lines is to enable it to adjust itself and the interendothelial space to environments. The interendothelial space is the seat of physiology of the peritoneum, for as the protoplasmic portion of the cell expands or contracts, it narrows or widens the interendothelial space.

9. So far as my experiments in interperitoneal injections and micro-

scopical examinations are concerned, the diaphragmatic serosa is the chief territory where solid particles are absorbed. However, both my experiments and microscopical examinations are too limited for any definite or final conclusions. The reasons for the diaphragmatic serosa being the only region where material is absorbed is given by Bizzozero, Salvioli and Muscatello as due to the anatomical fact that the membrana limitans possesses perforations only on the serosa of the diaphragm. Although the view that apertures are confined to the membrana limitans exclusively cannot be definitely settled, certainly the stomata vera do not appear any different on the diaphragm than they do in other regions.

10. The absorption of organic and inorganic finely divided material being confined chiefly, at least, to the diaphragmatic serosa, it seems that a stream must be directed toward the diaphragm, which may account for rapid deaths in perforative and other kinds of peritonitis. The idea of a current toward the diaphragm is based on the result of experiments, e. g., carmine or Berlin blue suspended in fluid and injected into the rabbit's peritoneum was found later in the subserous region of the diaphragm, especially in the large-branched connective tissue corpuscles and the lymph channels.

11. The view that the peritoneal serosa is normally a continuous sheet or surface without any apertures except when leucocytes force their way through the network of intercellular space apertures has been advanced and remains. I can not consider it in accord with experimental and microscopical evidence. Certainly the stomata vera found on the sheep's mesentery or the cisterna lymphatica magna of the frog are absolutely and distinctly anatomical structures and cannot be reasonably interpreted into merely temporary apertures produced by a few leucocytes forcing themselves through the interendothelial space. No number of leucocytes forcing their way through interendothelial space would leave behind an aperture lined by distinctly granular, polyhedral cells capable of being outlined by a microscope.

12. In careful examinations of the peritoneum, not only of man but also of the horse, dog, pig, cow, sheep, bird, rabbit, turtle, frog and embryos of man and of the pig, it was observed that the endothelia of the peritoneum was easily desquamated by trauma and inflammation. In many specimens of tubes, ovaries and uteri by immediate staining with Ag. NO<sub>3</sub>, it was found that the inflammation of the organs and the accompanying trauma of removal nearly always desquamated the endothelia so much that it destroyed the specimens for proper study. Severe inflammation desquamated almost every plate from its bed.

13. The structures located in the free surface of the peritoneal endothelia show powers of remarkably rapid absorption, and hence free



drainage of the abdominal cavity is the prophylaxis against invading septic peritonitis.

14. Beck experimentally demonstrated and confirmed the well-known clinical fact that the peritoneum absorbs material more than three times faster than the pleura. In opening bodies in autopsies it is well known that inflammatory pleuritic bands are far more numerous than inflammatory peritonitic bands. The reason that pleuritic bands are in excess of peritonitic bands is due to the slower absorptive power of the pleura. If the pleura or the peritoneum is given time to oppose the invasion or absorption of material, protective exudates arise. The more rapid absorptive powers of the peritoneum, over the pleura, is an important clinical fact and in all probability is due to the interendothelial structure, viz.: stomata vera and spuria and also its extensive interendothelial spaces. So that the stomata vera, the contractility of the protoplasm of the endothelial cell widening the interendothelial space and the stomata spuria may be looked on as important in the physiology of the peritoneum.

## CHAPTER IV.

### SUPERITONEAL TISSUE.

"Some books are to be tasted, others to be swallowed, and some few to be chewed and digested.—*Lord Bacon*."

The peritoneum itself consists of a single layer of flat endothelial plates so joined at their edges that they make almost a continuous membrane. This membrane is so thin that one cannot feel perhaps five or six layers between the fingers and thumb. It is so thin and transparent that very beautiful and perfect microscopical views of structures can be secured by taking the membrane from a recently-killed animal and mounting it in glycerine without any preparation whatever. All parts of the peritoneum, except the shiny, slippery, endothelial plates, its covering shingles, will be considered subperitoneal tissue. The subperitoneal tissue consists of (a) *membrana limitans*, a glass-like membrane, which resembles a soap bubble in its walls, (b) fibrous tissue, (c) elastic tissue, (d) areolar tissue, (e) wandering tissue-corpuscles, (f) fixed tissue corpuscles, (g) various kinds of cells, as vacuolate, branches, fibrillar cells, fat cells, and also nucleus and nerve cells. Now this subperitoneal tissue differs in the quantity of the above elements it contains according to the location from which it is chosen. We will choose a small bit of the subperitoneal tissue from the side of the *psoas* muscle just beneath the peritoneal membrane. Place the bit of tissue on a glass slide in a drop of water and tease it well with a couple of pins, after which absorb out the water and place a drop of glycerine on the under surface of a cover-glass in which to mount the specimen. Now begin the microscopical search. The first thing to note is bundles of fibrous tissue—tens of thousands of fine strands mostly existing in wavy bundles of variable thickness. This is known as white fibrous tissue. The wavy bundles are composed of silky fibres, so very fine that it requires a high power to see them. If one does not tease these subperitoneal tissues, the bundles run quite parallel, though they be wavy. But teased, the bundles and fibers show a confused mass. The fibers interlace, cross each other, pass under and over, and present a tangled network. Non-teased, it presents dense planes of fasciæ. Again, by careful study with the microscope, one can make out that these various wavy bundles are representative of different planes of tissue. This accords with the

gross anatomy, for one can split and resplit the subperitoneal tissue into many thin shiny planes. These shiny planes of mesoblastic tissue resemble the peritoneum. They shine, glisten, are white and reflect light. The "white fibrous" subperitoneal tissue serves as a bed and support for vessels and nerves. It holds them in their proper course and protects them from injury. There is no place in the body where white fibrous tissue can be more beautifully observed than in the subperitoneal region. It is almost primitive in its perfection. The fibrils in the subperitoneal tissue are very long. In microscopic specimens the bundles reach from one side of the field to the other.

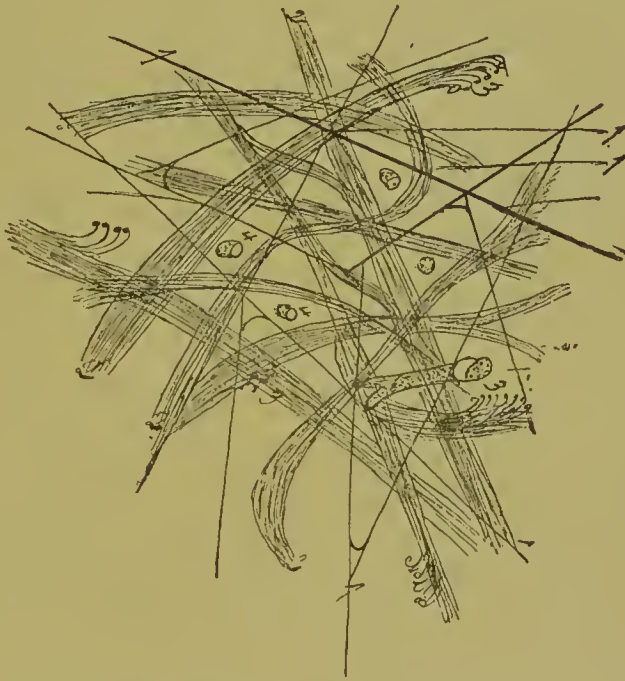


FIG. 67—(Author.) Human subserous tissue. 1, 1, a network of straight, elastic fibres. Note the triangle at point of anastomosis. 2, 2, wavy network of fibrous tissue. 3, 3, 3, show fibrous tissue curled up at the ends. 4, 4, granular cells. 5, branched cell with perhaps beginning vacuolation. This specimen was teased and mounted in glycerine.

Klein estimates them several inches long. The bundles are held together by an albuminous substance, semi-solid, which on boiling results in gelatin. White fibrous tissue in the peritoneum presents two varieties: (a) One variety is found especially in the omentum of man and dog and cat. It may be designated as reticulated fibrous tissue. The omentum is fenestrated. Holes appear in the omentum at varying periods after birth, and the fibrous tissue remains in wavy reticulated bundles, while large, clear spaces exist between the bundles. In these irregularly-shaped spaces no tissue of any kind exists. It has all been absorbed away. The process of atrophy is not well understood. I noted that this form of omental fenestrating begins in the human being



before two years. The reticulated bundles of fibrous tissue in the omentum are all entirely wrapped with endothelial cells, so that no fibrous tissue existed uncovered. The reticulated fibrous tissue is illustrated beautifully on young puppies or young children. (b) The other form of white fibrous tissue in the peritoneum exists in wavy bundles of varying size which interlace with each other in all directions and planes. The only difference in the two kinds of fibrous tissue in the peritoneum consists in its location and arrangement. The fibrils are white, and though they lack extensibility or elasticity in the ordinary sense, yet by teasing and stretching the bundles fly back or recur into wavy lines. The nature of the silky white fibres makes them an effectual support of adjacent organs. Are these long, silky, fine fibrils the drawn-out process of cells or the condensed, homogeneous, semi-fluid portion, primitive cell processes? It may also be noted that subserous white fibrous tissue exists chiefly in membranous planes or fasciæ. It is easy to secure these membranous planes or mesoblastic, subserous tissue by snipping out pieces from the broad ligament or along the psoas muscle and then to place the portion of tissue in water. As it swells up one can move it to and fro, when fine flocculent portions of extreme thinness may be seen floating in the water. These fine parts, single planes, can be snipped off with sharp scissors and immediately mounted in a drop of glycerine. I have examined with special care the subserous tissue in woman, and it can be obtained teased or not teased in the most typical condition. The white fibrous tissue in the broad ligament and adjacent to the bladder is rich and abundant. The fibres, if not teased, run quite parallel in the same fine thin planes, but if the tissue is teased out, the fibres show a dense mesh-work, a confused mass. Sometimes a long bundle of fibres may be seen as straight as an arrow passing across the whole field, but generally the silken, fibrous strands are wavy, owing no doubt to the rough handling. The amount of white fibrous tissue situated around the urino-genitary apparatus is absolutely enormous, so far as numbers of strands and bundles are concerned. The mobility of the genito-urinary organs is accounted for by the excessive amount of subserous tissue. These organs rest on beautiful beds of white areolar tissue which resemble newly-fallen snow or soft eider-down.

Among older histologists, it is interesting to note the strife as to what is the essential element of a serous membrane—viz., (a) endothelium, (b) fibrous tissue, or (c) elastic tissue. (The other variable elements they had not discovered or perhaps interpreted.) Bichat, who only lived thirty-one years (1771–1802), recognized the independence of serous membranes. He owed his suggestions to Pinel. Valentin (1810–1883) discovered the endothelium. Some claimed that

the serous fibres are the essential characteristic of the peritoneum, while others say that it is the elastic fibres, and still said some, "It is the endothelium." In 1851, Luschka (1820-1875) claimed the essential of the peritoneum to be "serous fibres"—i.e., the white fibrous bundles. How great men grope when in the dark, when microscopic light does not shine! But in the '40's came the sharp-eyed Henle (1809-1885), who saw things through a microscope, and said the essential constituent of the peritoneum is the endothelium, and his remarks remain yet unchanged.



FIG. 68—(Author.) A sketch of some elastic fibres after teasing from human subserous tissue near psoas, forty-eight years old; thirty hours after death mounted in glycerine (Oc. 2, obj. 8a, Reichert). In human subserous tissue the elastic fibres are very numerous. The teasing tore up the attachment of the base of the fibres, and hence they are irregular.



FIG. 69—(Author.) Drawn from omentum of a boy two years old. Ag. NO non-pencilled, non-fenestrated. Just at this spot the dots are few, but in closely adjacent fields the dots are very large and numerous. The cells are very irregular in shape and size. The drawing is from a whole trabecula. 1,1,1,1, ground substance, i. e., fibrous trabecula; 2, 2, endothelia; 3, stomata vera shimmering through below the endothelia; 4, dots, stomata spuria; 5, 5, 5, stomata (vera) open (Oc. 4, obj. 3, R.).

The white fibrous connective tissue of the peritoneum is characterized by pale, water-colored fibres, by sharp though dark-colored contours. The fibre has generally a straight course, sometimes it has wavy or more rarely a sinuous outline.

So far as I have examined, they are present everywhere in the peritoneum. They lie embedded in different planes, and the fibres of the same plane run quite in the same direction. The fibres of the various planes cross each other at all angles from 0 to 180 degrees. The interlacing fibres may make a wide mesh-work or a mesh-work so thick and dense as to be opaque to the microscope. Luschka says the thickness of the fibres varies from .001 millimetre to an immeasurable fineness.

I have noticed that the fibre is so fine that the addition of several hundred diameters to the microscope makes scarcely any perceptible changes; even when a single fibre traverses most of the microscopic field and finally divides into two, but little change in thickness may be observed with 400 diameters.

The quantity of fibrous bundles varies in different portions of the peritoneum. They are most numerous where they are needed. For example, where much motion is required, as adjacent to the genito-urinary organs and small intestines, and around contracting and expanding vessels, the silky strands of fibrils are very numerous and abundant. The physical properties of the white fibril of the peritoneum, then, are non-elasticity and slight expansibility. It runs in parallel courses of bundles or threads of almost immeasurable fineness. It forms thin planes, a network of broad and fine meshes, and the fibril anastomoses with others at very varying angles. The wavy, sinuous, and confused meshes of fibrils, generally seen through the microscope, are no doubt due to trauma and reagents; the limitations of the white fibril, that of subserous tissue, i. e., from the membrana limitans to the bed-structure of the subperitoneal tissue. The chemical characteristic of the white fibril is that certain reagents, as acetic acid, make it swell and lose its distinct contour. The fibril becomes more visible and can be examined in different relations. Boiling the fibril produces gelatin. Potassium and sodium salts also affect this fibril, as well as some other reagents, as aniline dyes. Microscopically, the fibril cannot be seen in too bright a light. To stain with carmine aids to see certain matters, but the most natural method is to mount fresh subserous tissue in glycerine.

The second predominant element found in subperitoneal tissue is the elastic fibre. I have examined this in man, the cat, dog, rat, guinea-pig, birds, pig, sheep, cow, horse, turtle, frog, rabbit, and other animals. The elastic fibres are easy to recognize by their straight course and wide-meshed network. They are highly refracting, and have a distinct and sharp outline. They are homogeneous and possess a uniform width throughout their length. A peculiar triangular outline may be observed at the junction of the fibres. Of course, a difference in thickness of the fibres may be observed where an elastic fibre branches off from a bundle. The elastic fibre may take a curved or wavy course, but the curves are generally wide and regular. The waves are not short or undulating like white fibres. The elastic fibres anastomose in reticulated manner. The meshes or network may be quite small or very large. In the rat and dog they form a dense network. The mesh-work is very strong and small in the rabbit. The mesh-work of elastic fibres is fine, dense, and enormous in quantity in the subserous tissue of the



human ligamentum latum. If the elastic fibres are loosened from their attachments by trauma, as, for example, by teasing, they assume a curled-up appearance. The broken ends will curl up like a hook and show a very irregular looping. The elastic fibres are so dense and numerous in the mesentery of a rabbit that it looks like reticulated or adenoid tissue. So far as the human is concerned, the subserous tissue adjacent to the bladder and genitals is densely packed with thicker and thinner bundles. The elastic fibres are a distinct, vast element, rivaling the white fibrous tissue in this region. This fact accounts for the capacity of the genital organs to alter their position and return to normal without loss of integrity. The elastic fibres account for the mobile

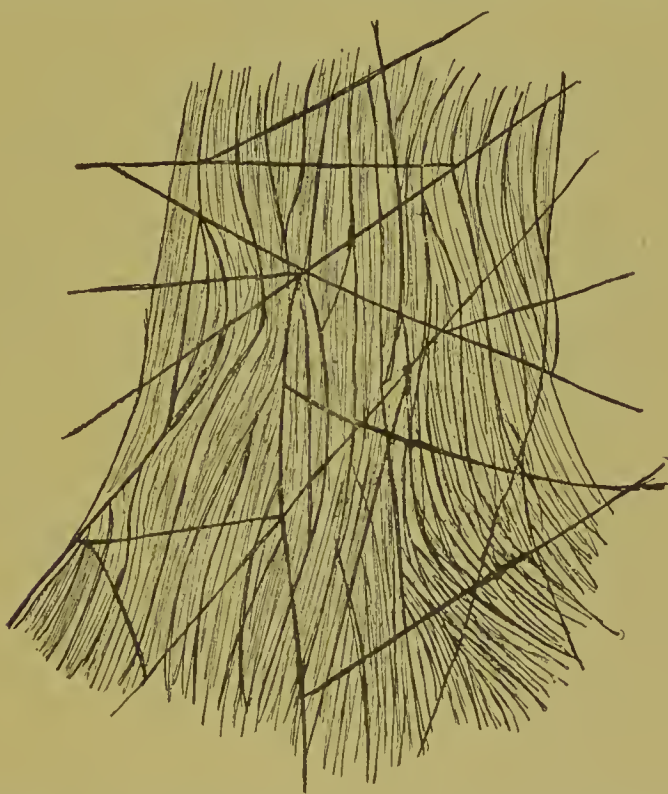


FIG. 70—(Author.) Human subserous tissue. It is here attempted to represent a single plane of mesoblastic fibrous and elastic tissue. It is mounted in glycerine, but was not teased. The black network is elastic fibres.

uterus to a large extent. The elastic elements in mesenterial supports, ligamenta peritonei, is a characteristic feature to allow distension and contraction of viscera and lessen shock in the sudden movements of animals. It also enables the subserous tissue to accommodate itself to the distension and contraction of blood and lymph vessels; still more to enable the viscera to adapt themselves to the varying condition of the abdominal walls and moving diaphragms.

The elastic fibres show their own significance from the anatomic fact that they exist in the subperitoneal tissue, as far as I have made

research, in proportion to the mobility of the viscus. Where an organ has a fixity to it, the elastic fibres exist in a minimum. In short, the wonderful contractility and expansibility of the peritoneum is due to elastic fibres. It adjusts itself to physiologic and pathologic conditions by elastic elements. In examining, microscopically, many portions of the serous membrane one can observe a variety of elastic tissue. (a)

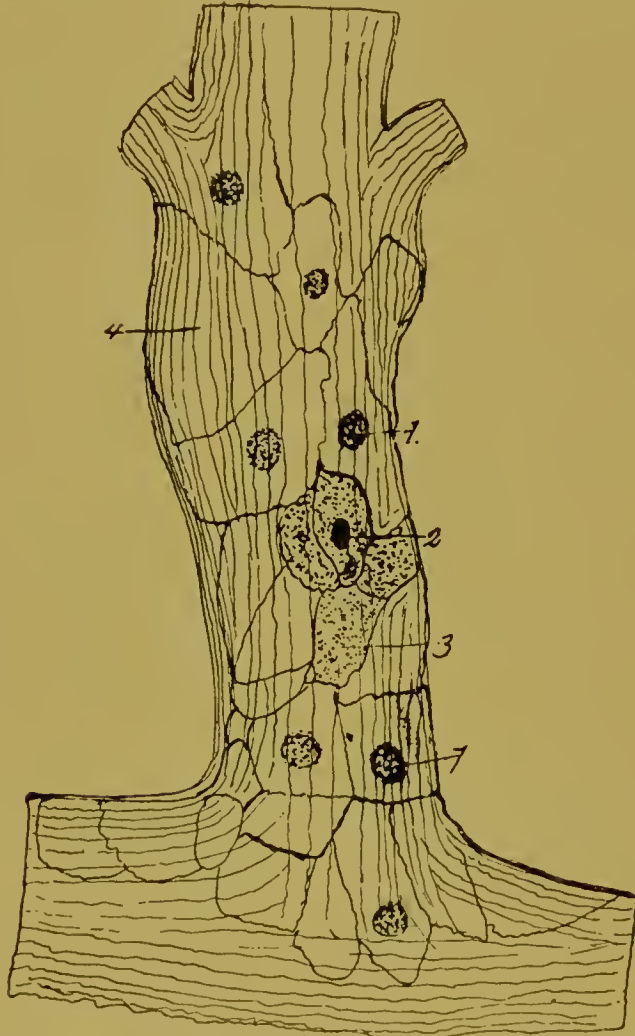


FIG. 71—(Author.) Drawn from a trabecula of adult human omentum majus (Oc. 4, ob. 3, R.). It shows a stoma verum of four granular germinating cells, and the underground substance. 1, 1, nuclei of endothelia; 2, points to nucleus of germinating endothelia. Some parts of the granular germinating endothelia of the stoma verum appear as if they were shimmering through the transparent endothelia. 3, germinating endothelium of the stoma verum; 4, endothelia covering the trabecula.

In some fields of the microscope we can note a long, fine fibre of elastic nature which never branches. It may take a large, sweeping curve or a short, spiral-like curve, but it always remains the same unbranched strand; it never splits. In teased preparations it may be irregularly curved or spiral from being loosed from its attachments.

(b) Another variety, which is abundant in the carnivora, especially the cat, rat, and also the rabbit, is characterized by the anatomical fact

of existing in bundles, and it sends branches out at various points. The bundles of elastic fibres may be of considerable thickness. They may send off branches, and these in turn send off more branches. This form of elastic fibres may be so abundant as to out-rival all other forms of tissue, especially in the mesenterium. It may be best observed in its anatomical relations by mounting a piece of mesentery in glycerine. It is not then disturbed by tearing or trauma, and one sees the two endothelial membranes and the *membrana mesenterii propria* undisturbed. This form of elastic fibre divides or branches so that at the point of division a peculiar triangle is observed. It is distributed irregularly in the subserous tissue.

(c) Another form assumes a kind of irregular meshwork. It con-

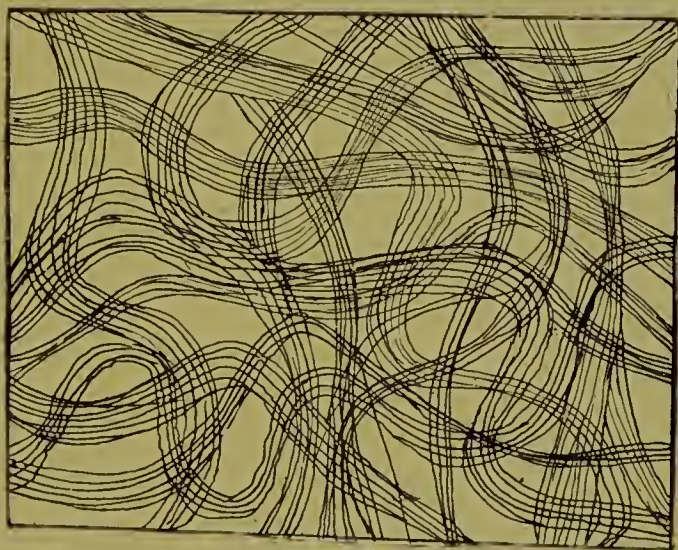


FIG. 72.—(Author.) Dog's mesentery in a mild solution of formalin; only the white fibrous tissues are drawn. Some are very much waved. Notice that the fibres all cross the whole length of the field. The fibres are so rich in number that no kind of drawings gives an adequate view. In this specimen no elastic fibre is represented. However, the elastic fibre is quite scarce here.

sists of quite fine fibres. Professor Hubert Luschka drew such a natural figure of such fibres forty-five years ago that I will recopy it to illustrate the elastic network. The forms b and c may not always be easily distinguished from each other on account of the peculiar way of branching. But the form c is distinctly more like a fish-net, while the variety b takes wider and more sweeping curves, and its mesh-work is more irregular. (d) A fourth form I will only mention, as I have not investigated it. It is the membranous form of elastic fibre, so well described by Henle. It is found especially in large arteries. Of course I might say that this membranous form of elastic fibre is almost exactly the same as others, except that the large bundles in the form of b have simply blended, leaving small or large holes between the wide bundles. In such a form I have studied it, but not in the large blood vessels.



Finally, it may be said that it is not always easy to distinguish a single isolated elastic fibre from a single isolated white fibre.

The fibrous connective tissue together with the elastic fibres constitute the mesenterial supports, *ligamentum peritonei*, the *membrana mesenterii propria*—that is, the white fibrous and elastic tissue is woven into web or membrane, which is the real neuro-vascular visceral support. In the mesentery this *membrana mesenterii propria*, consisting of elastic and white fibrous connective tissue, is faced on both sides by flat endothelia. The mesentery consists, then, of three parts—viz., (a) a layer of flat endothelia on each face and (b) a middle layer, the *membrana mesenterii propria*. The middle membrane of the mesentery supports the arteries, veins, lymphatics, and nerves, and retains them in anatomic and physiologic relations. The *membrana mesenterii propria* is the chief element in visceral support. In fact, the endothelial membrane facing each side is merely the whitewash of a wall or the veneer of a building, while the iron framework is the *membrana mesenterii propria*. The various proportions of white fibrous or elastic fibres which go to make up this *membrana mesenterii propria* characterize the different mesenteries of animals. The rabbit's *ligamentum peritonei* is characterized by much elastic fibre, and especially any mesentery which is called on to do much work. And any mesoblastic bed that is adjacent to a mobile organ is particularly noted for its predominant supply of elastic tissue.

Besides the two dominating elements of elastic and white fibres, there are other smaller and more irregular elements found in microscopic examinations. It is plain to note microscopically that the web woven by the elastic and fibrous strands extends like sheaths all along the vessels to the remotest parts of the body. This mesoblastic web of elastic and fibrous cords ensheathes or fixes the great blood vessels and nerves and lymphatics, especially of the abdomen. It supports in order to retain in relations the viscera. It is, in fact, the mesoblastic bed, the mesoderm, in which was originally developed the blood vessels and the lymph vessels. There are two views held as regard the origin of the white and elastic fibres. One view (Beale and Schultze) is that the protoplasm of one kind of embryonic cell is directly converted into fibrils while the protoplasm of another kind is converted into elastic fibres. Another view is that the white fibrils are deposited in the ground substance, while the elastic fibres (according to Ranvier) are from the fusion of granules or globules. These fusions result in the fine elastic fibres and elastic membranes. But still the small elements of subserous tissue demand attention.

First we may consider the granular cell. This cell consists generally of a round body which has a number of distinct granules in it.

The nucleus is round or oval. The cells are numerous near blood vessels, and are especially abundant in the subserous tissue of the broad ligament, which is quite vascular. I noticed that such often has its

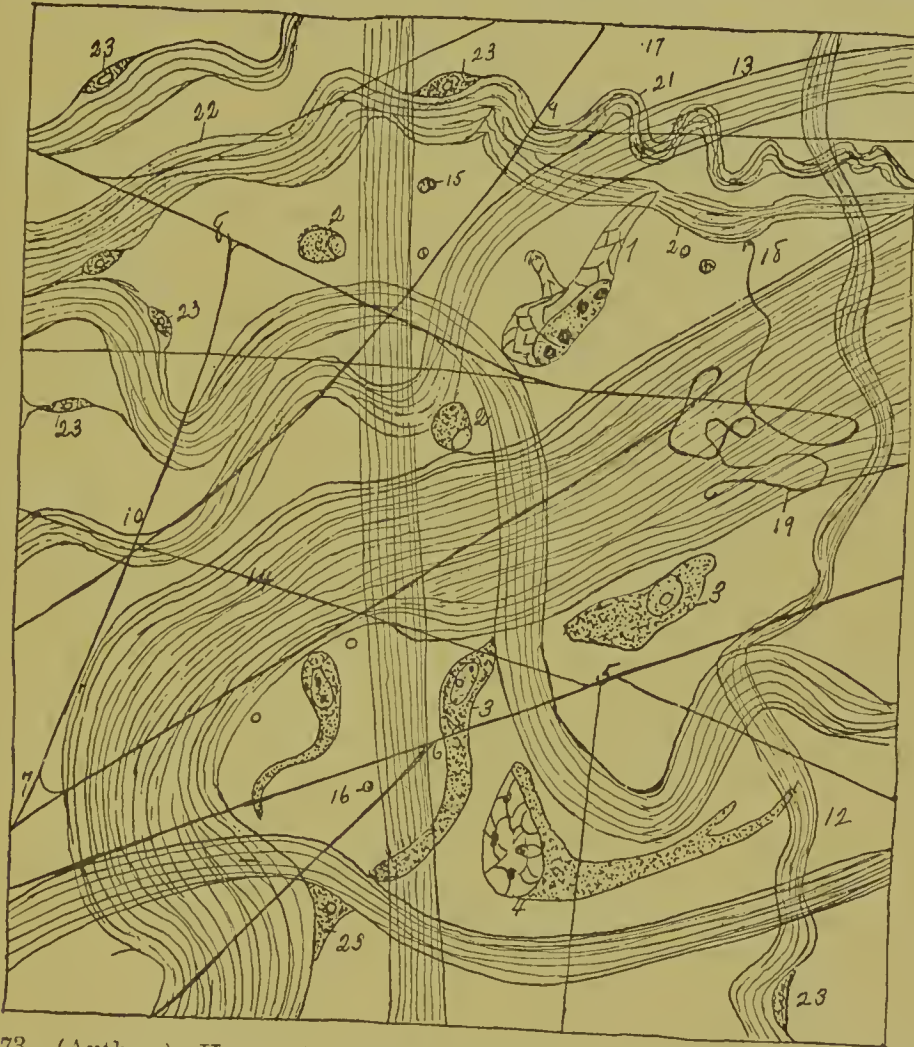


FIG. 73.—(Author.) Human broad ligament from a woman of fifty. The tissue was carefully cut from the cadaver, dead thirty hours, and placed in water. The subserous tissue is microscopically divisible into exceedingly thin mesoblastic planes, and these planes, almost invisible, float in the waving water. This specimen is cut from one of these very thin planes, and mounted in glycerine without being teased, hence the bundles of fibrous tissue are about parallel, of course, but an infinitesimal portion of the fibres is drawn. 1, vacuolated cell with oval nucleus and nucleoli; 2, 2, granular cells most abundant near blood vessels; 3, 3, flat or branched cell; 4, partially vacuolated cell. The flat, branched, or vacuolated cells are quite irregular elements. 5, 6, 7, 8, 9 and 10, represent a wide mesh-work of elastic fibres; 11, 12, 13, and 14, bundles of white fibrous tissue (non-teased); 15, 16 and 17, granular wandering, or lymph-cells; 18 and 19 show the curly endings of elastic fibres; 22 splits up into the straight bundle, 20, and the wavy one 21; 23, 23, 23, 23, connective tissue corpuscle.

nucleus situated towards one side, excentrically, while the granules collect especially towards the one side. The granule cell is very variable in shape and size and even in the distinctness of its granules. But, in general, the granule cell I found abundant in the subserous tissue of

man, rabbit, and frog. Such cells are said to stain well with eosin and some aniline dyes. However, the cell is plainly recognized by mounting freshly-teased preparations of subserous tissue.

The fixed connective tissue corpuscle must be well studied in subserous tissue by a high power. The connective tissue cell consists of a body and a nucleus toward its center. The body may appear flat, stellate, or with branched processes. The connective tissue corpuscle suffers many changes on account of the participation in the intercellular tissue. In primitive condition, as in Wharton's jelly or the umbilical cord, the connective tissue cell has a formidable size. But with growth-changes the body assumes a smaller outline, and the protoplasmic processes contract to fine thread-like fibres, and finally the small nucleus is contained in a thin-walled body. As far as I can see, the granular and connective tissue cells may be occasionally confused with each other. Both cells vary considerably in size.

In the subserous tissue there is a peculiar cell known as plasma or vacuolated cell. These cells were especially described by Waldeyer. They may be found fairly constant and frequently in teased preparations. These cells are of an indefinite form, round, spindle-shaped, and some possess projecting processes. There are vacuolations in the cells which distinguish them from other cells. Again, a part of the cell may be vacuolated while the other portion may be quite granular. I have noted that the nucleus is quite large sometimes and of an elongated oval.

The flat, lamellar, or branched cell found in the subserous tissue belongs to the connective tissue cell. These are the various forms with varied processes projecting from either end. The nucleus is large, round, or oval with a nucleolus. Again, in contact with the larger connective tissue elements may be seen large numbers of small, round, or ovoid masses. These are lymph corpuscles, and on account of their capacity to change their locality, are called wandering cells. These wandering cells appear of various sizes. The difference in size may be owing to the plane of vision or to their more or less flattened shape. In some specimens of subserous tissue, well teased, I have noted much larger numbers of wandering cells than others. Why one specimen of subserous tissue is characterized by more wandering cells than another is not clear.

The fibro-elastic strands underlying the peritoneum are the binding, supporting, and accommodating element. They exist in quantity according to the necessities of the viscera—fixed viscera demands a small amount, while movable organs require a large amount, especially of the elastic.

The practical application of the fine structure of the subperitoneal



tissue to medicine and surgery is important. The chief subperitoneal tissue belongs to the dorsal wall, and in this region will play the chief role of disease. The subperitoneal tissue supports viscera in their bed. It is liable to inflammation, primarily, but especially as a secondary process—an extension from diseased viscera. The chief role which the subperitoneal tissue plays is its relation to pus. Retroperitoneal abscess is just beginning to be understood. These abscesses are not infrequent of occurrence and nearly always present very complicated



FIG. 74.—(Author.) Drawn from boy's omentum majus, two years old. Ag. NO<sub>3</sub>.  $\frac{1}{2}$  per cent. applied and partly pencilled. This figure is drawn close to a field which has enormous-sized endothelial cells. Where the endothelia are brushed off, the interendothelial lines are nearly all left undisturbed. 1, 1, Nuclei, some endothelia containing a double nucleus; 2, 2, stomata vera; 3, endothelia; 4, 4, 4, 4, trabeculae of three different planes; 5, 5, spots in the nucleus—nuclei.

Note how endothelia vary in size and outline in this omentum majus of the same individual.

symptoms. We noted that the tissue was composed of exceedingly fine laminae, of thin plates of mesoblastic planes. Any fluid can find its way slowly between these thin planes of tissue. No doubt the reason that pus penetrates so universally in all directions in the subperitoneal tissue is because it is composed of fibro-elastic planes which are separable by slowly pressing fluids. One can microscopically divide and tear this fine tissue into numerous fine, transparent sheets. The pus will press these planes apart in the direction of least resistance. The pus derived from diseased pelvic organs takes many directions because

it has no definite barrier. The pus may pass up over the ilium over the pubis, through the peritoneum, along the rectum, along the large blood vessels on the side of the vagina or through the sacral foramen. The pus of appendicitis, when it arrives in the subperitoneal tissue, splits the planes of mesoblastic tissue towards the liver, because the subperitoneal tissue along the psoas muscle is abundant, and where it is abundant its planes are more easily separated. In fact, I have found in most bodies that the mesoblastic planes of subserous tissue are always the most easy of cleavage. Besides the planes of easy cleavage in mesoblastic tissue directing pus and fluids between its laminae there is another application in regard to it. It is known that when fat accumulates in this subserous tissue that its droplets gradually collect within the capsule of a connective tissue corpuscle or cell. The fat droplets slowly coalesce and grow, expanding

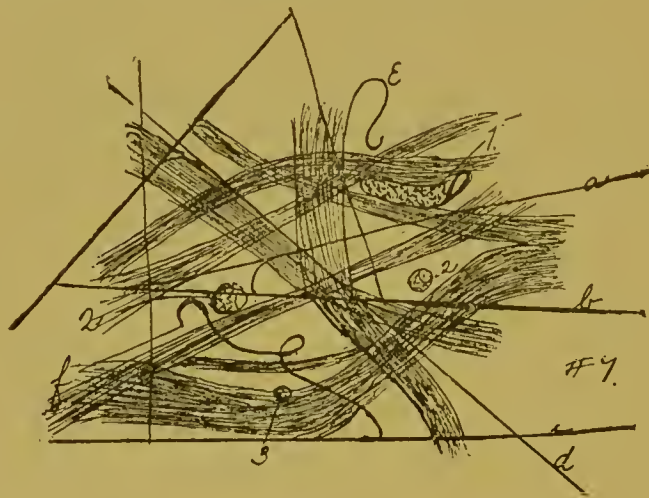


FIG. 75—(Author.) Human subserous tissue selected near the psoas muscle. 1, A branched cell; 2, 2, granular cells; a, b, c and d, elastic fibres showing a wide network of straight elastic fibres. At e and f the elastic fibres curled up by being teased—i. e., broken from their attachments. This tissue was teased, and as soon as elastic fibres are broken they spring into curls or loops. 3, Wandering lymph-corpuscle.

the connective tissue cell or capsule enormously. The fat globule expands until the little nucleus of the connective cell is scarcely visible in its thin wall. This fatty tissue accumulates or aggregates in the genito-urinary region, and especially in the omentum majus, i. e., in reticulated fibrous tissue until it assumes vast bulks. Now, this fatty tissue or oil globules often suddenly disappear. Now the bulk of areolar tissue, i. e., connective tissue with corpuscles expanded with oil, has been expanded so long that either its elasticity does not resume its normal function or it is not able to readjust itself so rapidly. The contracting connective tissue capsules cannot force the adjacent viscera to return and resume the old relations as before. In such cases the kidney wobbles about and with time becomes more or less movable. Its bed is too large. The same occurs with the uterus, which simply glides out of

the enlarged vaginal outlet; especially will the uterus glide out of the vaginal outlet if it has become retroverted and intra-abdominal pressure strikes it on its anterior surface. In sacculation and absorption of fat oil globules, doubtless the number of connective tissue corpuscles which stored the fat globules remains the same, though on sudden absorption

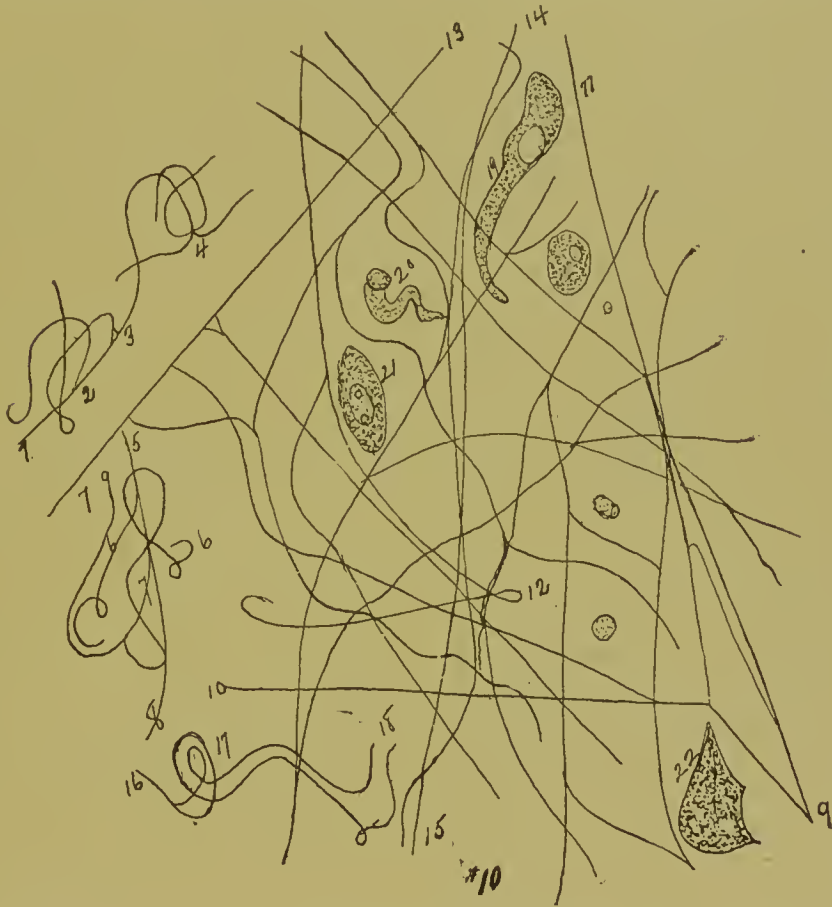


FIG. 76.—(Author.) Human elastic subserous tissue teased and mounted in glycerine. 1, 2, 3, and 4 show an elastic trunk which branches and curls up at the ends; 5, 6, 7, and 8 show other elastic fibre endings; 9, 10 and 11, straight fibres; 12, curled elastic fibres; 13, an elastic trunk; 14 and 15, straight non-branched elastic fibres; 16, trunk; 17 and 18, branches of elastic fibre; 19, 20, 21 and 22, various kinds of cells lying among the elastic fibres; 22, appears dividing into two cells with two nuclei.

the fat globule, the connective tissue corpuscle, has lost its function. It has forgotten to resume its old business. It cannot return to the normal, for it has lost its integrity. Perhaps the sudden absorption of fat shocked the periphery of the nerve supplying the connective tissue corpuscle for a season. However, we may note the two important applications of subperitoneal tissue, viz.: (a) it consists of fine planes which permit of cleavage by slowly pressing fluids; (b) by sudden absorption of fat it becomes impaired in function.

To sum up, we note that subserous tissue consists of (1) in quantity,



white fibrous connective tissue; (2) elastic fibres; (3) connective tissue corpuscles; (4) wandering cells; (5) flat, lamellar, or branching cells; (6) granular cells; (7) vacuolated or plasma cells. That its planes of cleavage allow fluids to press in universal directions, and that rapid absorption of fat from subserous tissue elongates and impairs visceral supports.

The *membrana limitans* or basement membrane is very limited in quantity, but stretches over a wide area.

#### THE DIAPHRAGM AND CENTRUM TENDINEUM.

The diaphragm is the chief characteristic of mammalian myology. The amphibia and animals below them in scale of structure have no diaphragm. The first distinct trace of it may be found in crocodile and bird, where the muscular fibres which are concerned in its formation arise from ribs. Even animals as high in the ascending scale as birds have an imperfect diaphragm; it does not separate the lungs and abdomen of aves completely. In fact, several descriptions have appeared as to what is considered a diaphragm in birds. A complete diaphragm which arises from the vertebral column, ribs and sternum is a mammalian property only. The exact mode of the formation of the muscle is not fully known. The mammalian diaphragm is probably homologous to the so-called diaphragm of other vertebrates. The mammalian diaphragm is supplied by the phrenic nerves, which arise from the fourth, fifth and sixth cervical nerves and course along the lateral borders of the heart in contact with the pericardium to supply chiefly the anterior primitive portion of the diaphragm. In the human species it receives a sympathetic branch from the inferior cervical ganglion. Some of the lower intercostal nerves pass to the midriff. Besides, the diaphragm receives sympathetic branches from the abdominal brain along the phrenic arteries—the phrenic plexus. Originally the body cavity extended in the embryo from the visceral arches to the pelvic cavity; in the mammalian embryo the pericardio-thoracic cavity begins to be distinctly marked off from the future abdominal cavity by a transverse fold. This transverse fold begins at the vertebral and lateral wall, projects median-ward and dorsal-ward into the primitive pleuro-peritoneal cavity. This fold marks the course which the terminal part of the omphalo-mesenteric vein takes in order to reach the heart.

Oscar Hertwig says: "Subsequently there is found imbedded in the transverse fold all of the venous trunks which empty into the arterial sinus of the heart, i. e., the omphalo-mesenteric and umbilical vein with the ducts of Cuvier which collect the blood from the walls of the trunk." From this view it would result that the transverse fold—the incipient diaphragm—is intimately connected with the development of the veins. Similar folds are produced in the peritoneum by blood vessels as the

plica-duodeno-jejunalis, the folds due to the arteriæ hypogastricæ and umbilical vein. It is known as the septum transversum, or as Uskow named it, "massa transversum."

The primary diaphragm really belongs to the heart, as it consisted originally of projecting folds through which were conducted blood vessels to the great fluid or blood center—the heart. It may be noticed that the ventral part, i. e., the primitive or original part, of the diaphragm is the older, and this explains why the two phrenic nerves chiefly supply the primitive or anterior portion. The muscular portion of the diaphragm gradually projects from the lateral and dorsal aspects toward the central portion of the body cavity between the growing liver and sinus venosus. A final fusion results between the primitive and second-

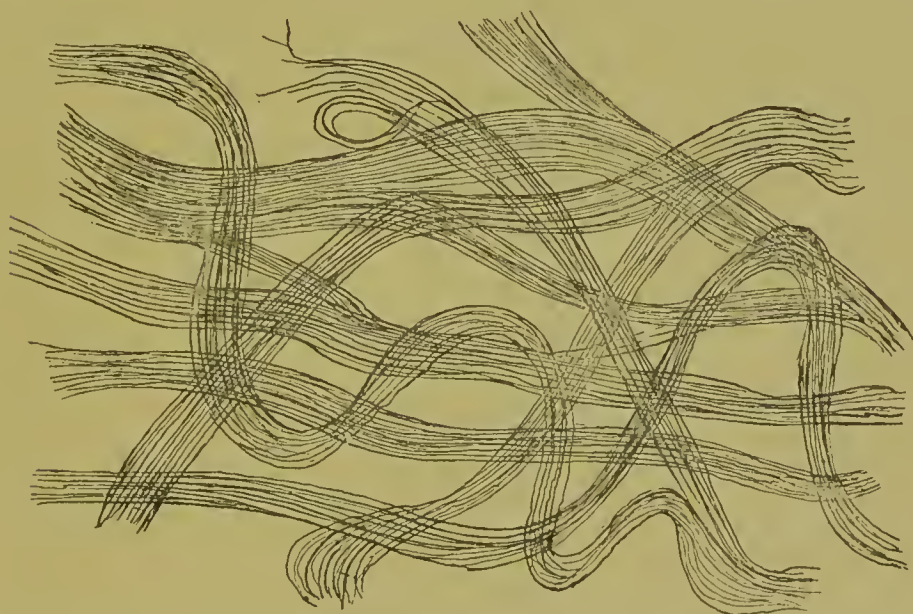


FIG. 77—(Author) Human subserous tissue, drawn after teasing, mounted in glycerine (Oc. 2, obj. 8a, Reichert). When the subserous tissue is not teased the fibres of the same plane are nearly all parallel.

ary portion of the diaphragm. The hepatic and diaphragmatic development is inseparably connected early in embryonic life. But first the pericardial cavity is completely closed and there remains two tubular cavities projecting from the peritoneal cavity bilaterally up to the visceral clefts. His called these cavities thoracic prolongations of the abdominal cavity—a very suitable name. Into these original peritoneal prolongations jut out the lung structures, growing from the vertebral wall of the intestinal tube. Later in embryonic life the pericardial cavity is closed and occupies the chief ventral side of the embryo, the thoracic cavities now closed occupy the dorsal side, while the rapidly growing peritoneal cavity occupies the posterior portion of the embryo, all three cavities distinctly divided off by the characteristic mammalian muscle of the diaphragm. The pericardial sac first closes from above

by the forward projection of the ducts of Cuvier, the primitive diaphragm forming the lower portion of the pericardial sac. Finally the edges of the projecting folds, due to the ducts of Cuvier, fuse and the pericardial sac is formed. The remains of Cuvier's ducts is the superior vena cava. From the dorsal and lateral walls of the trunk project folds, known as the pillars of Uskow, which fuse with the original septum transversum which was thrown into a fold by the veins which course to empty their contents into the heart. The diaphragm has an older ventral part supplied by the phrenic nerves and a younger dorsal part. The pericardial sac is of enormous size in the embryo while the two narrow, lateral tubular sacs hold the rudimentary lungs which are very small from non-use and slight blood supply. But with further neutral growth of the lungs they detach more and more the wall of the pericardium from the diaphragm and from the lateral walls of the thoracic cavity, thus increasing the pleural diaphragmatic surface. Again, the liver is gradually separated from the primary or ventral portion of the diaphragm by the peritoneum growing over and becoming adherent to the upper surface of the liver, only leaving a small portion of the upper surface uncovered by peritoneum. This uncovered liver surface is bounded by the basal margins of the coronary ligaments. The characteristic mammalian muscle serves mechanically as, 1, a partition to divide the peritoneal cavity from the pleuro-pericardial cavity; 2, to fix the pericardial sac and 3, as a floor to support the pleuro-peritoneal endothelium. Anatomically it is so designed by origin and insertion as to aid respiration, allowing lung expansion and aiding contraction. It permits of much adjustability of pleura-peritoneal organs. It accommodates itself to the varying size of adjacent viscera. Physiologically the diaphragm is a vast and active absorbent. It is a filter, a sieve for peritoneal fluids (and also to some degree for pleural fluids). A considerable portion of the central part of the diaphragm of most animals is of a tendinous or aponeurotic nature. In some mammals there are several tendinous portions separated by muscular and connective tissue ridges.

In cetacea the centrum tendineum is almost obsolete.

In the study of the histology and physiology of the mammalian diaphragm the chief attention is nearly always confined to the centrum tendineum. My studies were mainly concerned with the diaphragm of man, the dog, rabbit and guinea-pig. The most convenient animal on which to study the central tendon of the diaphragm is the one which possesses a central tendon so thin and transparent that it will require no section for microscopic examination. The cheapest, most accessible or perhaps the most satisfactory animal on which to pursue histologic and physiologic study of the centrum tendineum is the rabbit. The centrum tendineum of this animal consists essentially of four layers,



viz.: First, there is a layer of parallel radiating tendinous bundles, passing from the region of the vertebral column toward the costal arches. This tendinous layer lies on the posterior or abdominal side of the diaphragm. In the intertendinous spaces or clefts of this layer, long irregular interstitial or lymph spaces exist; with the naked eye one can observe the radiating tendinous ridges, especially by stretching the tendon. In physiologic experiments it is in the intertendinous spaces that

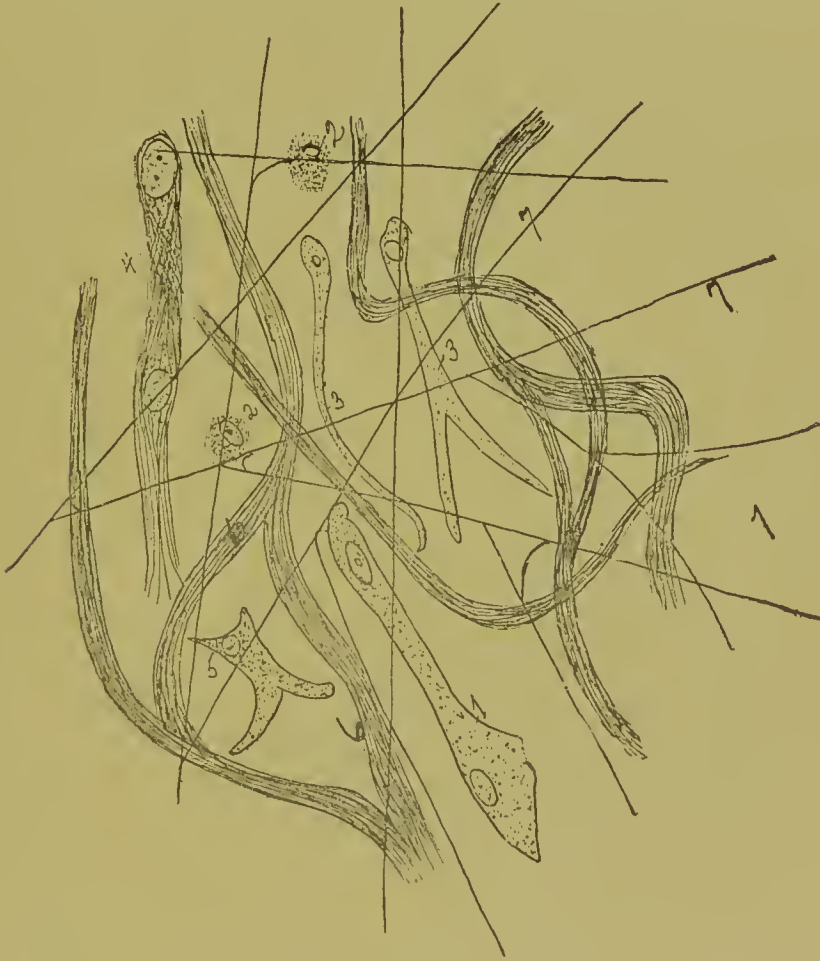


FIG. 78.—(Author.) Drawn from human subserous tissue near psoas. It was teased a little, but did not break up the elastic mesh-work which forms its bed. 1, branched cell with two nuclei; 2, 2, granular cells; 3, 3, branched cell; 4, fibrillated cell; 5, branched cell with nucleus; 6, white fibrous bundles; 7, 7, elastic fibres.

the chief accumulations of colored granules are deposited and really seen by the eye or tracing lens.

The second layer of the centrum tendineum is the circular or that lying on the pleural side of the diaphragm. It is quite uniformly developed, but at localized points there appears to be an excessive amount collected. These two tendinous layers, the radiating and circular, may be readily dissected from each other.

A third layer of the centrum tendineum is the pleura serosa lying on

the circular tendinous layer. It is composed of endothelia interrupted by stomata vera and spuria. It lies in contact with the lung.

A fourth layer enters into the centrum tendineum, the abdominal



FIG. 79—(Author.) A trabecula of human omentum 48 years old, dead thirty hours when it was stained with Ag. NO<sub>3</sub>. (Oc. 2, obj. 8a, R.) Observe how irregular the endothelia are. No stomata vera, spuria or intraendothelial appeared. 1, nucleus; 2, endothelia. The human omentum majus is covered with endothelia which varies very much in shape and size.

serosa. This is an endothelial membrane composed of flattened connective tissue cells, so placed edge to edge as to produce a membrane

which is interrupted only by stomata vera and spuria. The diaphragmatic abdominal endothelia rest on the basement membrane, the *membrana limitans*, which is perforated by groups of small openings. The histology and physiology of the above four layers of the *centrum tendineum* will engage our attention both experimentally and microscopically.

Histologically, the *centrum tendineum* has engaged the attention of original investigators for over thirty-five years. Von Recklinghausen began 1861 by showing that particles suspended in fluid and injected into the peritoneum passed through the serosa of the *centrum tendineum* and were liberally deposited in the lymphatics of the diaphragm. He used milk, oil, cinnabar, Chinese tea, etc. His pupils Pia Foa and Radjewsky continued his researches. Chrzozozinsky, and his pupil, Affannasiew (1867), did some excellent work on the dia-



FIG. 80.—Luschka, 1851. It illustrates how elastic fibres will form a mesh-work resembling a net. It also shows how the superficial and deep layers of the elastic planes will blend and anastomose with one another. The drawing is taken from synovial membrane.

phragmatic peritoneum. The most extensive and far-reaching labor on the *centrum tendineum* since the epoch-making experiments of Von Recklinghausen were the researches of Ludwig and his pupils, Schweigger-Seidel, Dogiel and Dybkowsky in the Leipsic Physiologic Institute in 1864-67. Those laborers asserted that the peritoneum is a lymph sac. The Russians, Lawdowsky and Kolossow, did excellent work. The Italians, Bizzozero, Salvioli, Maffucci and Muscatello, were progressive workers, and Bizzozero announced in 1874 that the *membrana limitans* of the *centrum tendineum* was perforated by groups of openings. The excellent labors of the Frenchmen are well known, as those of Ranvier, Dubar, Remy, Tourneaux, Hermann.



The Englishmen, Klein and Burdon-Sanderson, produced meritorious works. The combined labors of these investigators point to the diaphragm or centrum tendineum as the significant locality of physiologic activity of the peritoneum.

The serosa of the peritoneal side of the centrum tendineum presents special features requiring careful attention. If a rabbit's diaphragm be stained in situ with 2 per cent. solution of  $\text{Ag. NO}_3$  and removed without trauma and mounted in glycerine, we observe the endothelia and their corresponding dark network of interendothelial spaces; interendothelial substance is discarded. At the common junction of several endothelial cells may be seen structures which are designated stomata vera, while situated along the interendothelial spaces are structures known as stomata spuria. Again, another distinctive characteristic of the centrum tendineum is presented in the microscopic field which consists of parallel dark and light spaces. These dark and light strips radiate from the vertebral region toward the costal arches and correspond to the tendinous bundles and intertendinous spaces. The distinctive feature of the peritoneal serosa of the centrum tendineum is that directly over the radiating tendinous bundles the endothelia are large, regular and possess relatively few stomata vera, while the endothelia covering the light or intertendinous spaces are small, quite regular and possess very numerous stomata vera. Occasionally the large, regular endothelia may not only stretch as usual over the tendon bundles, but in irregular distanced localities even bridge all the way across the lymphatic intertendinous channels, taking the place of the small, irregular endothelial cells which cover the lymph spaces. In intertendinous spaces the stomata are arranged in rows chiefly occupying the central portion of the space. The light spaces are intertendinous lymph channels, while the dark spaces represent non-transparent tendinous bundles. The intertendinous lymph channels are irregular in shape and size, possess lateral bulgings or sinuses and run parallel with the bundles of tendons. The size of the intertendinous lymph channels varies according to the amount of fluid in them. They measure according to Schweigger and Dogiel, from 0.06 mm. to 0.12 mm., i. e.; they vary one-half in size.

The stomata vera of the peritoneal serosa of the central tendon, located chiefly in the intertendinous lymph spaces, are vertical canals connecting directly the peritoneal cavity with the subperitoneal lymph channels. It must be admitted that there is an uncertainty in the examination of the peritoneum whether the stomata vera really correspond to holes in the serous membrane or not. Yet this uncertainty becomes less as one actually observes that, by injecting fluid holding in suspension solid particles into the abdominal cavity, the solid particles will pass in vast numbers into the subperitoneal lymphatics of the cen-

tral tendon of the diaphragm in a few minutes. The stomata vera are lined by granular, polyhedral, nucleated cells. The vertical canal has varying lengths. Sometimes it may only be as long as the thickness of the peritoneal layer, plus that of the subperitoneal lymph vessel wall, or the canal may pass down obliquely. Again, the vertical canal may pass down through a wide lymph capillary field to connect a deep subserous lymph space. In such a case the vertical lymph channel is really invaginated by the lymph space through which it passes.

It is true the stomata vera are irregular in distribution, shape, size and number, but that can hardly be considered sufficient proof against

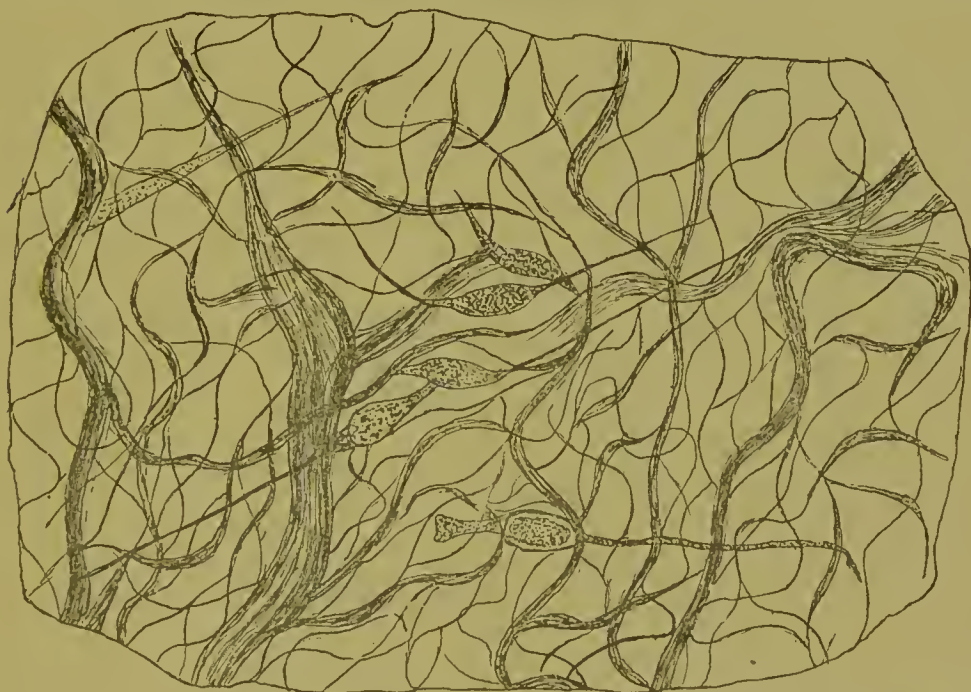


FIG. 81.—Rollett in Stricker's Handbook, 1871. It represents the connective tissue fibrils of a five-months-old embryo. It also shows the elongated fusi-form cells.

their existence. Schweigger-Seidel's theory that the stomata vera are surrounded merely by the nuclei of the adjacent endothelial cells must be abandoned, as it does not correspond with the observed facts. One can see the usual nucleus in the endothelial plate, which surrounds the stomata vera in its accustomed locality. In examining specimens of the central tendineum the stomata may be observed open or closed, the mouth may represent a curved line as is easily observed on the lymphatic cisterna magna of the frog. Klein adds another kind of stomata which are mere breaks or discontinuity in the endothelia leading into some lymph space which is only covered by a single layer of endothelium. Such stomata are not lined by granular, polyhedral, nucleated cells. The application of a solution of  $\text{Ag. NO}_3$  to the stomata vera of the diaphragm produces in them a rich brown color, owing to the amount of

precipitable albumen they contain. The stomata vera or vertical lymph channels are lined by a special layer of endothelial cells, more or less polyhedral in shape, consisting of granular protoplasm. The expansion and contraction of the granular nucleated cells which line the vertical lymph channels control the lumen of the stomata vera. They have a sort of sphincter so as to control the flow of peritoneal fluids. Ranvier called the stomata vera lymph wells and claimed that the so-called granular cells lining them were leucocytes. They are doubtless localities for reproduction of endothelial cells to replace worn-out or dying comrades. My experiments seem to show that the Berlin blue particles may be found passing through, deposited in the vertical lymph channels.

On the interendothelial spaces of the centrum tendineum there exist after the application of silver nitrate solution dark spots, rings, ovals, thin rings with large light centers or thick rings with small light centers or very irregular masses. These structures are known as stomata spuria. Virchow called them lymphoid cells; Oedmansson, Von Recklinghausen and others called them connective tissue corpuscles projecting upward between the endothelial plates. I am quite well convinced by experiments that leucocytes wander from the lymph spaces below to gain entrance to the peritoneal cavity.

The interendothelial space in the centrum tendineum originally was called interendothelial substance, fluid, semi-fluid or cement substance. I have discarded all names for the word interendothelial space. By the use of osmic acid as a fixation agent, and silver nitrate and tannin as reduction agents, we can dissolve the interendothelial space into two parallel lines with an intervening light space crossed transversely by anastomotic protoplasmic processes. The two parallel lines on the borders of the cover-plate and the transverse anastomotic processes are the protoplasmic processes which bind the endothelial cells into colonies and groups. The anastomotic processes are thin and fine at the surface but increase in thickness and numbers as they descend from the surface. This interendothelial space gives ample room for contraction and expansion of endothelial cells, contraction of the cells, and elongate and thin the protoplasmic anastomotic process, and the expansion causes the reverse condition. It thus can regulate peritoneal fluid currents.

The endothelium of the centrum tendineum consists of a cover-plate (Kolossow, Ranvier), an indurated, hardened, metamorphized portion of protoplasm which is fixed by protoplasmic processes to the subjacent protoplasm, but the lateral edges have little or no connection with adjacent fellow endothelial cover-plates. The best animals to study to observe the cover-plates are the frog and turtle. The portion of the endothelial cell immediately beneath the cover-plate is the real living protoplasmic



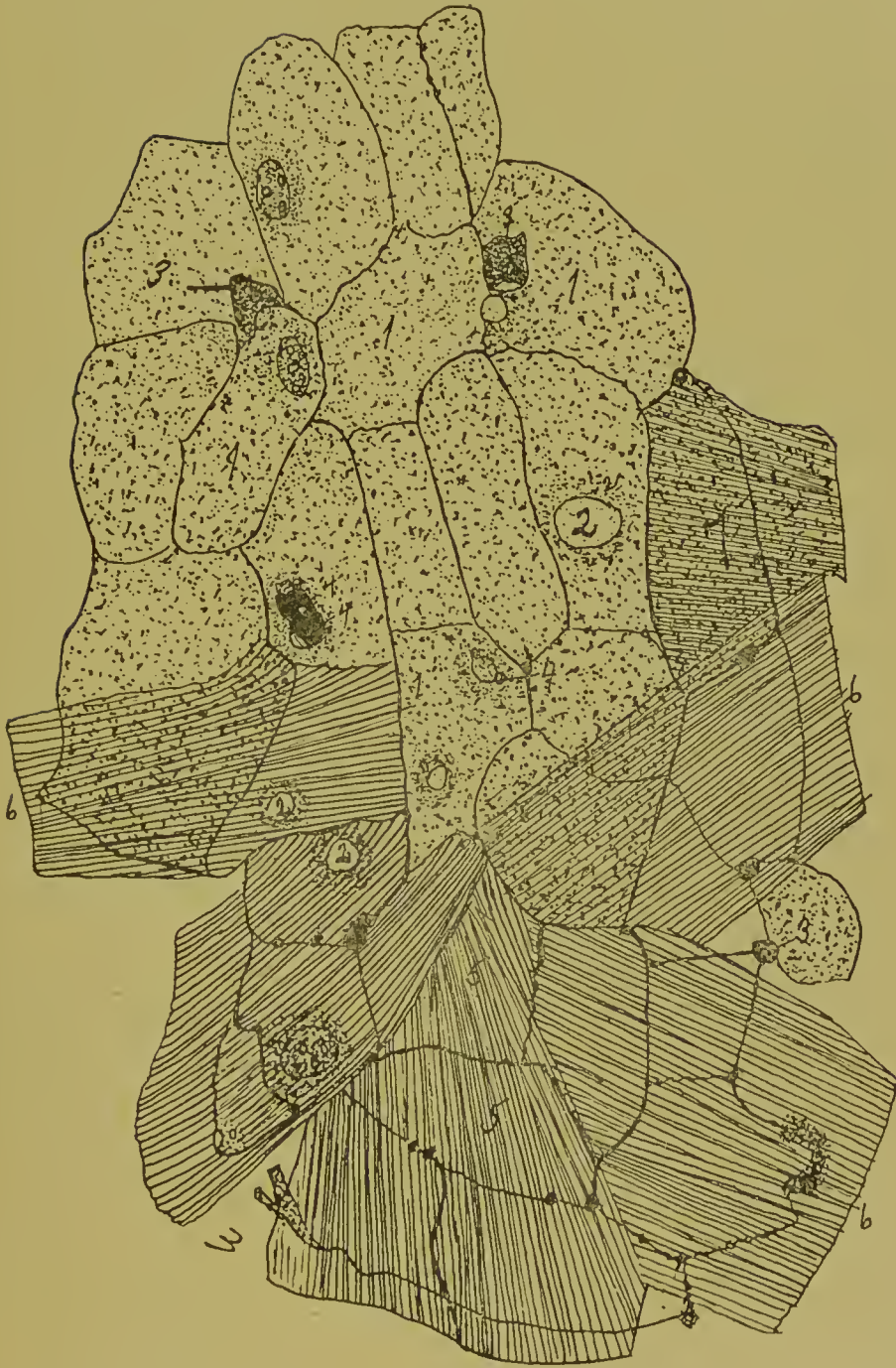


FIG. 82.—(Author.) Drawn from omentum majus of a boy, two years old (Oc. 2, obj. 8a, Reichert). Part of the endothelia were pencilled off. Where some of the endothelia were pencilled off (7) there is a kind of pepper and salt condition, albuminous precipitate of silver. Also the interendothelial lines on the pencilled portion show black dots. 1, 1, Endothelia non-pencilled; 5, 5, places where the endothelia were pencilled off; Ag.  $\text{NO}_3$ ,  $\frac{1}{2}$  per cent. applied; 6, 6, 6, bundles of subserous tissue or trabeculae; 2, 2, 2, nuclei; 4, 4, 4, intra-endothelial stomata or precipitated matter; 3, 3, 3, stomata vera. This drawing lies adjacent to a field of regular endothelia, among which are innumerable stomata vera.

essential part of the endothelial cell which contains the nucleus. It is contractile and expansive and connected to its fellows in colonies by anastomotic protoplasmic processes. The pleural serosa situated on the anterior or upper surface of the centrum tendineum is similar in structure to the peritoneal serosa situated on the under or posterior surface of the tendon.

We note then that the centrum tendineum has two layers of serosa and two tendinous layers. The elements of the serosa are the endothelial plates, stomata vera et spuria and interendothelial space, which we



FIG. 83.—(Author.) Drawn from omentum majus of a boy, two years old. Ag.  $\text{NO}_3$ .  $\frac{1}{2}$  per cent. applied. Part of the field was pencilled, and the endothelia are partly gone, showing the subserous tissue. Ground substance with no endothelia; 2, 2, endothelia; 3, 3, closed stomata vera, black dots; 4, 4, open stomata vera; 5, group of seven endothelial cells; 6, 6, shows two portions of cells almost constricted off—i.e., into additional endothelial cells; 7, shows another group of endothelia, six in number; 5 and 7 are likely stomata in stages of development.



FIG. 84.—(Author.) Gastrosplenic omentum of dog, 12 weeks old. Fenestrated with very distinct endothelia, nucleated endothelia, very irregular, but they wrap themselves around trabeculae. The omentum of the dog is fenestrated like a man's. A nucleus is apt to lie at division of trabeculae. No stomata vera or spuria appear in this field. 3 shows an endothelial cell broken away from its attachments; 2, nucleus, and 1 a fragment of cell or precipitated debris.

have discussed sufficiently to expose the views of their structure and significance. We will now discuss the fine membrane on which the endothelia rests, the peculiarity of which is that it is perforated by groups of apertures in the serosa of the abdominal side of the centrum tendineum. It is known as the membrana limitans.

The membrana limitans is a transparent, glassy, fibrillar-like membrane situated beneath the peritoneal endothelia. The earliest account



of this membrane at command is a well-written article by Brinton, in Todd's Encyclopedia, 1847, under another name, "basement membrane." Todd and Bowman describe it as a "continuous transparent membrane of excessive tenuity and homogeneous or nearly so." Goodsir (1847) also described it, but he noted that it could be separated into its component cells which were of a rhomboid and extremely flattened shape.



FIG. 85.—(Handbook of Phys. Lab., Vol. II., 1873.) Pleural side of rabbit's diaphragm brushed and silvered. (a) Lymph vessels with valves, passing over into (b), lymph capillaries. (c) Islands of ground substance showing the canalicular system. The lymph vessels are recognized by their sinuous outline. Observe the lymph vessels are connected with the lymph capillary and the capillaries with the canalicular system.

Goodsir named it the "germinal membrane." Many examinations of this subject were made fifty years ago, with the result that vigorous denials of its existence were asserted. Brinton himself could not confirm Todd and Bowman's investigations. Arnold, in his "Handbuch der Anatomie," Freiburg, 1844, p. 216, calls it "a finely granular, fibreless ground substance." There can be no doubt that the basement



membrane of Todd and Bowman and the granular, fibreless substance of Arnold are one and the same membrane. In 1850, Koelliker, in his "Mikroskopische Anatomie," could not fully confirm the basement membrane, but acknowledged that beneath the endothelial layer there appeared a homogeneous element which was similar to a membrane. Henle, in 1840, "Ueber Serosen der Haute," in Froriep's "Notizen," demonstrated this membrane as intercellular substance of connective tissue, and again as formless germinating material. Luschka, in 1851, in "Die Structur der Serosen Haute des Menschen," calls it by various names, as structureless connective material, almost completely homogeneous, of glass-like transparency, smooth or very finely striped lamella. He says it is clear and shining and has the appearance of lightly ground glass. It may be noted that but little could be added to make Luschka's structureless material exactly the same as the differently termed *membrana limitans* of today. He says this structureless material is found in all the serous membranes between its fibrous elements. The far-famed Reichert who, in 1845, first proposed the term connective substance, considered this material intercellular substance, which is finally transformed into a membrane possessing rudiments of the original cell elements. Luschka incidentally remarks that Todd and Bowman found occasion to call their structureless membrane the basement membrane.

In 1873 Bizzozero, who studied extensively the peritoneum, established the less distinctly seen object of older authors as a definite, recognized *membrana limitans*. However, it is the same identical membrane of Arnold, Henle, Todd and Bowman, Luschka, Reichert, Goodsir, Koelliker and others. What brought Bizzozero into prominence is not reaffirming the existence of the *membrana limitans*, but the announcement that the *membrana limitans* is perforated by apertures on the diaphragmatic serosa. This significant discovery is the only explanation so far offered which explains why the finely divided, colored granules are so rapidly carried into the lymph channels of the diaphragmatic serosa when injected into the peritoneal cavity. The diaphragm is the chief region of absorption of the material injected into the peritoneum, because the *membrana limitans* is perforated only over the diaphragm.

The *membrana limitans* has been described in animals by Wadd and others. Bizzozero asserted that it contained no cells, that it is simply finely striped. Acetic acid applied to it makes it swell and become invisible from transparency. I have made long search as to the location of the perforations and so far have never seen the *membrana limitans* perforated outside of the *centrum tendineum*. Almost all the perforations of the *membrana limitans* found have been sit-

uated toward the line of junction of the centrum tendineum and the muscular portion of the diaphragm, i. e., toward the costal margin of the central tendon. My best specimens so far are from the human species and the dog. In these the membrane appeared as thin as that of a soap bubble with peculiar fine striations running parallel to each other, resembling glass ground in one direction only. The groups of perforations in the membrana limitans

in the human species were apparently larger than in the dog, and contained numbers of pores. In one case there appeared to be 80 to 100 pores in one group. In the dog I seldom saw groups containing more than 40 to 50, but



FIG. 86.—(Author.) Drawn from peritoneal side of rabbit's centrum tendineum to illustrate a small capillary distinctly invaginated in the vast lymph spaces through which it courses. The diaphragm was silvered and the simple handling of the diaphragm during an experiment sufficed to desquamate the free peritoneal endothelium so that one can readily see the blood vessel with its long spindle-shaped endothelia coursing through the vast lymph field recognized on each side by their sinuous endothelia. The lymph spaces, of course, pass over the blood vessels, but that is omitted to avoid confusion of endothelia. 1, 2, blood vessel; 3, 4, their nuclei; 5, stomata verum; 6, 7, 8, 9, sinuous endothelia of the lymph spaces; 10, 11, their nuclei; 11, 12 and 13, stomata vera of lymph spaces.

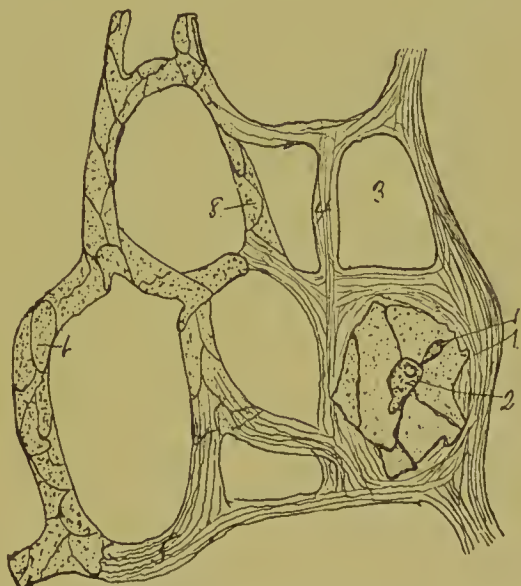


FIG. 87.—(Author.) Sketch made from omentum majus of a boy two years old. Ag.  $\text{NO}_3$ .  $\frac{1}{2}$  per cent. applied. It is already well trabeculated and reticulated. Part of the trabeculae are covered with endothelia, 8 and 6; 5, trabeculae of subserous tissue not covered with endothelia; 3, space absorbed—i. e., the endothelia and subserous tissue have atrophied completely; 1, the group of 6 endothelia shows a stomata verum. It shows the trabeculated condition at two years old.

oftener much less. The distribution of the groups of apertures was similar in man and dog. The group of pores resembled in distribution somewhat a vast herd of sheep in a wide pasture with larger and smaller groups here and there, with but a few stragglers existing between. The distribution of the groups of pores might be compared to the groupings of the gonococcus in a specimen.

Irregularity of number, size and shape of groups characterized the

distribution of pores on the peritoneal serosa of the centrum tendineum. The shapes of the pores are chiefly round and oval. The best specimens on which to find the pores are those preserved several days in Muller's fluid or preserved 12 to 24 hours in water, subsequently gently washing off the endothelia. In some specimens the pores could be observed through the serosa of the central tendon. So far in our work we have not found the pores in the center of the centrum tendineum, but always toward the periphery. The outline of the perforation or pore is always sharp and distinct. The circumference of the pore seems to take on the silver stain actively, making it appear quite distinct. Where the pores are located as Muscatello notes, the membrana limitans appear to be quite adherent to the adjacent tissue. As regards the ease with which the pores in membrana limitans may be demonstrated, I must demur to the idea that it is certain or easy of execution, for one may attempt to find them on many specimens before success results. It is easy to observe that the membrana limitans is thicker and thinner in different localities of the peritoneum.

The membrana limitans is a very fine, thin connective tissue layer on which the endothelia rest. It is a finely granular, or better, a finely fibrillar or striped membrane. It contains no cells. It has a watery or glass-like transparency, which is plainly observed when the endothelia are fallen off or very lightly brushed off. As Bizzozero and Salvioli have shown, the membrane is not exactly alike over all the parts of the peritoneum, but is perforated over the diaphragm. They say that the perforations of the membrana limitans are situated on the zona tendinea and the zona peritendinea of the diaphragm. Bizzozero and Salvioli assert that the pores of the membrana limitans have a diameter from four to sixteen mm. and a round or oval circumference. They occur in groups of fifty to sixty, of irregular egg-shaped form; the pores correspond to the meshes of the fibrous connective tissue. I have examined this glass-like membrane, the membrana limitans, in various parts of the peritoneum, as the diaphragm, omenta, mesenterium, ligamentum latum, and there is no doubt about one's ability to see the membrane when the endothelial layers have been carefully brushed off, but we can not always be sure to find it, and it is not easy to isolate. Again, to find the pores of the membrana limitans which Bizzozero and Salvioli say exist only on the zona tendinea and the zona peritendinea of the diaphragm is not at all easy, at least so far as my researches are concerned. It is rather to be said that it is an uncertain process to find the pores. Muscatello made examinations of the membrana limitans on the ligamentum latum of the serous covering of bowel, stomach, liver, spleen, pancreas, uterus and anterior abdominal wall and the diaphragm. He found that the membrana limitans, as Bizzozero and Sal-



violi have asserted, is perforated on the diaphragm. In no other place could he find pores in it. The method Bizzozero recommends to isolate the membrana limitans is to place large pieces of serous membrane several days in Muller's fluid and then 12 to 24 hours in equal parts of water and alcohol. Now the endothelia are brushed off with a pencil or washed off with a stream of water. One then tries to seize the membrane with a forceps so it may be isolated. The membrane is then spread out and colored with eosin or acid fuchsin, when one can exam-

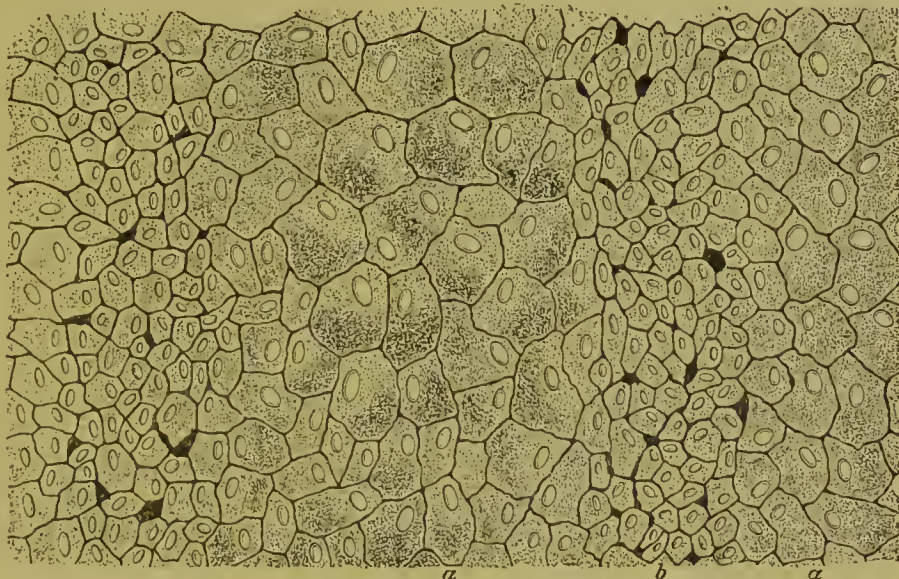


FIG. 88.—(Klein, Handbook of Phys. Lab., Vol. II., 1873.) Abdominal surface of centrum tendineum of rabbit intensely colored with silver. (a) Endothelium of the serosa where no lymph vessel is seen. (b) The same, showing an interfascicular lymph channel underlying the endothelium, in which a capillary lymph vessel runs. Note that the endothelia are smaller and more irregular between the tendons. The dark spots are interpreted by some as stomata. The nuclei are clear. The endothelia over two tendons and two intertendinous lymph spaces are shown. The endothelia over the tendons are large and regular, while the endothelia over the intertendinous lymph channels are smaller and more irregular.

ine it in glycerine and water. Muscatello notes, as all others who have examined the membrane, that it is a fine fibrillar structure. It is a membrane of a continuous uninterrupted surface with no pores except on the diaphragm. In my examinations of the membrana limitans I will here record a phenomenon, which few authors note, and that is if the endothelia are brushed off very lightly, or better, washed off, one can occasionally observe small pits or depressions in the membrana limitans. These pits or depressions are simply the places where the endothelial cells once were.

The only other author known to me as mentioning the fact is Muscatello. The membrana limitans is generally thicker over solid organs, such as the liver and uterus. However, one generally finds the membrane on the diaphragm with as much certainty as anywhere. Still, I

agree with Muscatello that one can often find it on the bowel as well. By careful observation one can often see endothelial cells, connective cells of fiber clinging to the membrana limitans at points where it was forcibly torn away. Again, in places it is so intimately associated with the underlying connective tissue that it can scarcely be separated, and a little too strong penciling ruptures it in various directions.

The membrana limitans is a thin sheet of tissue, a continuous membrane on which rests the protoplasmic portions of the endothelial cells. According to Bizzozzero, Salvioli, Muscatello and my own researches it



FIG. 89.—(After Peter Nikolsky, 1889.) Represents a portion of peritoneum from the surface of the cisterna lymphatica magna of a male frog (abdominal side). It will be noticed that inside of the ring of protoplasmic bodies in each structure there are other larger and more square protoplasmic bodies. Schweigger-Seidel claimed that the ring of roundish protoplasmic bodies were the nuclei of the adjacent radiating endothelial cells. I think Schweigger-Seidel's proposition is definitely disproved.

is perforated only on the diaphragmatic serosa, and hence arose the explanation of the fact that the diaphragm is the chief point of absorption of the peritoneum. The reason that the mesenterium will remain distended with air when blown up, as Bichat recorded it, is because the membrana limitans is not perforated on the mesenterium.

From considerable research and quite a number of experiments on the peritoneum of rabbits (and other animals), it appears that there is a current directed toward the diaphragm.

So far it appears the diaphragmatic membrana limitans alone pos-



sesses pores and is the chief locality of the peritoneum which possesses power to absorb and deposit the colored granules into the subjacent lymph channels. Dubar, Remy and Maffuci assert that there are

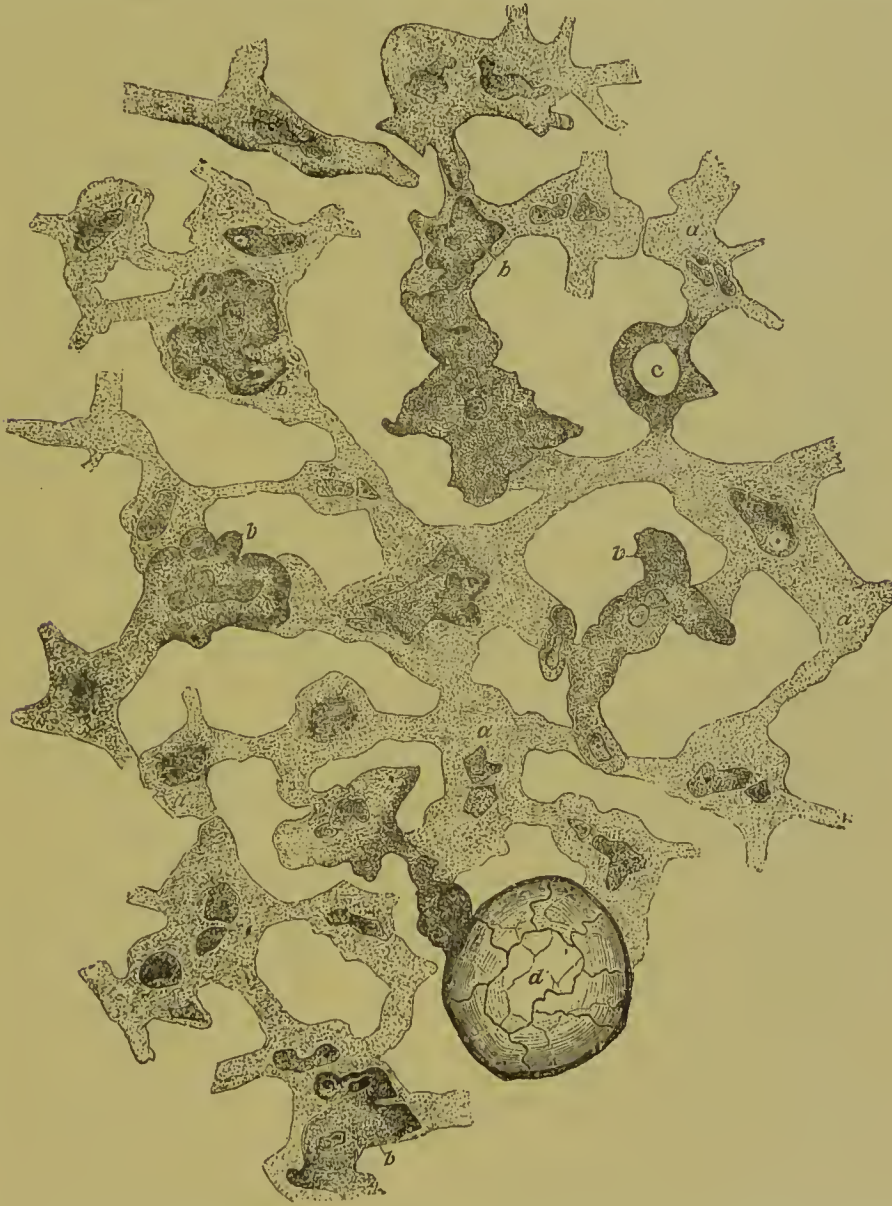


FIG. 90.—(After Handbook for Phys. Lab., Vol. II., 1873.) Preparation of surface of omentum of rabbit, pencilled and treated with silver. (a) The flat branched cells of the canicular system are visible as finely granular structures; their nuclei are sharply defined, in several places are seen in the act of dividing. (b) Migratory cells, some of which are free, while others grow out of the fat cells of the canicular system, like buds: in one of the latter the formation of a vacuole is seen at c, d. d, a vacuole cell, the wall of which is already changed into endothelial elements. (Oc. 3, obj. 9. Immersion.) This specimen shows well the ground substance.

other localities of the peritoneum for rapid absorption, but this has not been extensively confirmed. I have noted absorption of colored granules in the centrum tendineum, gastro-splenic omentum and about the omentum of the pylorus. The strange reason for the diaphragmatic



membrana limitans being the only portion perforated has received no adequate explanation so far. But the diaphragm is an organ of motion and a bed of lymphatics and perhaps the pores are a remnant of the original aperture between the pleuro-peritoneal cavity.

The significance of the perforation of the diaphragmatic membrana limitans and the stream of fluid in the abdominal cavity directed toward it is a practical matter of peritonitis, for it is very suggestive of drainage.

1. In my experiments on animals it was distinctly evident that the region of the diaphragm (including to some extent the omentum minus and root of the omentum majus), was the chief region of physiologic (and ultimate pathologic) activity. The swarm of leucocytes told the story. The particles of Berlin blue almost entirely passed into the vast lymph bed of the diaphragm. With time the particles pass into mesenteric glands and viscera, but this is entirely secondary to the passage into the lymph channels of the diaphragm.

2. It has been found that in puerperal peritonitis the serosa and lymph channels of the diaphragm are intensely inflamed as first demonstrated many years ago by Recklinghausen.

The centrum tendineum is a vast system of lymphatic channels. The credit of making the foregoing views known is due to Dr. Daniel Von Recklinghausen, now professor of pathologic anatomy at Strasburg. He demonstrated this fact by vast personal labors and many experiments, aided by his introduction of the use of silver salts. Ludwig, Schweigger-Seidel, Dybkowsky, Klein and others perfected his labors. The very reason that the centrum tendineum has had so much attention is because it is a bed of lymphatics and through it peritoneal fluids will pass carrying with them various kinds of solid particles. The following are the methods to demonstrate the diaphragmatic lymphatics:

1. By placing a very thin piece of the centrum tendineum of a small rabbit stained with silver nitrate and mounted in glycerine under the microscope, we may be able to observe the valved trunks and the non-valved capillary lymphatics. The serosa is sufficiently transparent to allow the lymphatics to be noted beneath it.

2. By brushing or penciling the abdominal serosa, but especially the pleural serosa of the centrum tendineum, with a piece of cotton on a toothpick or a camel's-hair brush and then subsequently pouring on the brushed surface 25 per cent. solution of silver nitrate for a few minutes, one can observe in specimens mounted in glycerine well defined lymphatic vessels and capillaries.

3. To inject into the abdominal cavity of a living animal (rabbit) fluid holding in suspension solid colored granules, subsequently (from 10 minutes to 24 hours) killing the animal, carefully cutting out the dia-

phragm, brushing its surface and silvering it. Mount small pieces in glycerine when the valvate lymph trunks and capillaries are plainly visible containing the colored granules.

4. The lymphatics may be demonstrated by carefully cutting out a diaphragm of an animal dead as long as twenty-four hours, silvering it and allowing it to remain one-half to several hours in a solution con-

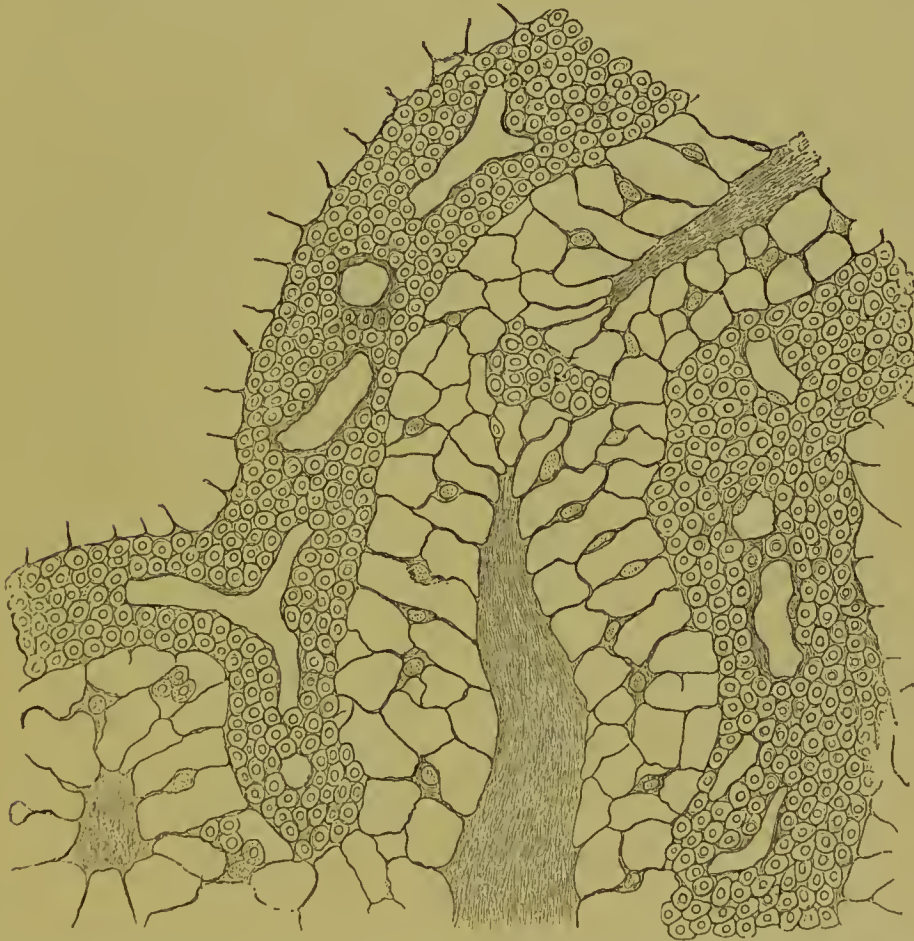


FIG. 91.—(After Handbook for Phys. Lab., 1873.) Section of the medullary substance of mesenteric gland of ox, which has been hardened in Muller's liquid and then partially shaken. The figure shows the lymphatic cylinders containing blood vessels, surrounded by closely packed lymph corpuscles, the finely fibrous trabeculae, and the system of cells between them. The blank spaces between the trabeculae and the cylinders represent the system of lymph sinuses, the lymph corpuscles of which have for the most part been shaken out. (Oc. 3, obj. 8. Tube not drawn out.)

taining colored granules of Berlin blue. Mounted in glycerine it shows the lymphatics containing the colored granules.

5. Inject into a recently dead animal (rabbit) a fluid containing Berlin blue, in half an hour cut out the diaphragm, silver, mount in glycerine, whence one may observe the lymphatics, capillaries and valvate trunks.

6. The lymphatics of the centrum tendineum fill better if artificial respiration be kept up for ten to fifteen minutes. The peritoneum of

the just-killed dog and rabbit absorbs fluid and solid particles about as rapidly as that of the living animal.

In every method one may expect frequent failures from trauma, i. e., dragging or too much brushing and occasionally too little brushing. After very many trials and experiments to demonstrate the lymphatics of the centrum tendineum, we would give the following simple directions: Kill a rabbit, open the chest and abdomen with as little trauma as possible. Wind a little cotton on a toothpick and brush the abdominal side of the centrum tendineum with the cotton, wet in the animal's serum or distilled water, gently two to three times. Then pour over the tendon in situ 2 per cent. solution of silver nitrate for three to five minutes. Serve the pleural surface of the tendon exactly similar. Now, with the most exquisite gentleness, cut out the diaphragm and place it in distilled water in the sunlight for a few hours. Snip off small bits with sharp scissors and mount them in glycerine. Preserve the whole specimen in Muller's fluid or 10 per cent. of formaline. For preservation the specimen should be dessicated with alcohol, however a very short time—one minute—or it will dissolve out the silver lines, a half-minute in oil of cloves and mount in xylol balsam. If one desires to have a clear nucleus, the specimen should be colored with hematoxylin for one or two minutes, and if a beautiful color is desired for the ground substance, the subendothelial tissue, a very dilute alcoholic solution of eosin for about a minute will perhaps be sufficient.

A curious but significant feature of the centrum tendineum is that where the pericardial sac lies on the tendon, lymphatics fail. There would be no particular physiologic object in having the centrum tendineum sieve-like immediately under the pericardium where it is adherent to the tendon, for the peritoneal fluids would then only pour into the pericardial sac.

The recognition of the lymphatics in the centrum tendineum after the application is easy and perfectly certain. We notice two kinds, 1, lymphatic trunks with distinctly visible valves, and 2, various kinds of capillaries and lymph spaces. By far the most typical location is on the pleural serosa of the tendon. It does not appear that the dog's lymphatic system is so well developed as that of a rabbit or guinea-pig, hence, for the purpose of demonstrating, the lymphatics of the latter animals should be chosen. Under the microscope the lymphatic trunk consists of a distinct wall, composed of sinuous or spindle-shaped, nucleated endothelium, which is beautifully brought out by the silver stain. The individual endothelial cell forming the lymph vessel wall may be enormously long, perhaps its major diameter may exceed its minor diameter by ten-fold, showing a very narrow and long spindle-shape, or the endothelia may be very irregular in shape and possess a



sinuous outline similar to the cranial suture. The vigorous emptying and filling of the lymph vessels give elongated shape to the endothelia as it does in blood vessels. It appears that the endothelia of the lymph vessels possess stomata vera and spuria similar to the peritoneal serosa; however, this is denied by some investigators. The interendothelial space is just the same as that of the peritoneum, i. e., consists of two dark parallel lines crossed transversely by anastomotic protoplasmic pro-



FIG. 92.--(After Handbook for Phys. Lab., Vol. II., 1873.) Centrum tendineum of rabbit, seen from the abdominal side. Berlin blue had been introduced into the peritoneum by "natural injection." (b) Straight interfasicleular lymphatics between the bundles of tendon of the abdominal side. (a) Lymph vessels of the pleural side, showing the valves, with corresponding dilations. The last lymph vessels are as completely injected as the first (Oc. 3, obj. 4. Tube not drawn out.) It is rare to secure lymph vessels so full by the "natural" or "physiologic" injection.

cesses. But the most significant and characteristic feature of the lymph trunks are the valves which appear on the vessels at very short intervals. The valves are a fold of the vessel wall and pass generally entirely across the wall. Frequently at the valves the endothelia change their course from a longitudinal to a transverse direction. Behind the valve the lymphatic trunk bulges out and one can observe a succession of such dilations and constrictions of the vessels. In fact, on the pleural surface of the centrum tendineum the lymph vessels have so

many valves that they resemble a succession of flasks placed one after the other. Sometimes the valves have a beautiful curve, and where the valve exists the vessel is enormously dilated. Vast numbers of lymphatic trunks exist in the centrum tendineum. The lymph trunks are very much wider than blood vessels, and if one is puzzled to decide whether the vessels be for blood or lymph, all that is necessary is to trace the vessels to a valve which will decide in favor of a lymph trunk. Again, in blood vessels as large as an ordinary lymph trunk one is able to observe longitudinal or circular muscular fibres; besides, the contour of a blood vessel is even while that of the lymph trunk is usually irregular.

The lymphatic spaces or capillaries of the centrum tendineum cover vast areas; in fact, in some specimens which happen to be well silvered and quite transparent, it appears that the whole of the centrum tendineum except where the heart rests and the periphery of the tendon or peritendinea is simply a bed or space of lymphatics. The lymph capillaries or spaces in the central tendon are characterized by sinuous spindle-shaped, or irregular nucleated endothelia. The word sinuous generally best expresses the shape of the endothelia. The capillary lymphatic system of the central tendon is vast, and one may frequently note the lymphatic capillaries by looking through the transparent endothelia of the pleural or peritoneal serosa. The lymphatic capillaries and trunks are seen to be connected and all stand in open communication with the peritoneal cavity. Several layers of lymphatic vessels lie in the centrum tendineum, such as the capillaries immediately under the pleural or peritoneal serosa, the vast intertendinous lymph spaces, the straight lymphatics, the deep lymphatics and those straight lymphatics which pass between the tendon bundles to connect the systems of lymphatics of the pleural and peritoneal side of the central tendon.

The distribution of the lymphatics in the centrum tendineum requires some time to determine experimentally and microscopically. The latter method I practiced extensively and found the trunks of lymphatics to pass, 1, toward the costal periphery of the tendon, 2, toward the mammary vessels ventrally, and, 3, toward the dorsal part of the tendon to reach the thoracic duct. The chief field for the lymph capillaries is toward the central portion of the tendon and the vessels gradually widen toward the periphery, finally terminating in four large lymphatic trunks, two dorsal-ward to the thoracic duct, and two ventral-ward to the mammary vessels.

In the physiologic method of demonstrating the lymphatics of the centrum tendineum we inject into the peritoneum of rabbits especially (sometimes employing dogs and guinea-pigs) a solution holding in suspension particles of Berlin blue, killing them from a few minutes to fif-



teen hours later. Shortly after such an injection into a rabbit's peritoneum, say ten to fifteen minutes, the centrum tendineum presents a brilliant display of radiating blue lines passing from the dorsal region

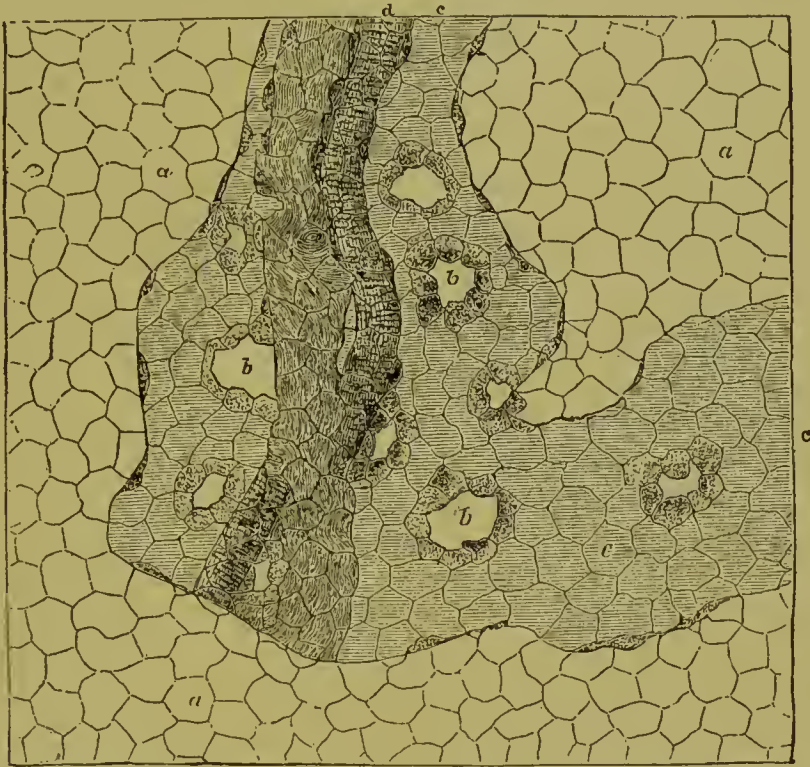


FIG. 93.—(After Handbook for Phys. Lab., Vol. II., 1873.) Silver preparation from centrum tendineum of rabbit. (c) A wide lymph vessel which can be seen through the surface endothelium (a). An artery (d), and a nerve trunk (e), pass through the lymph vessel (peri-vascular lymph vessel) (c), and within the field of vision are ten distinctly open true stomata (b). The surface endothelium bordering the stomata is germinating. P. 112. (Oe. 3, obj. 5.)

of the tendon toward its periphery in the intertendinous spaces. If the tendon is placed between the eye and strong sunlight the blue lines are very apparent. With a strong lens the lines are still more visible. It must be remembered that in some experiments unaccountable failures arise, as we have to do with factors of peritoneal absorption not yet understood. For example, I took a dog of about seven pounds weight and injected 100 cubic centimeters of fluid (composed of Na.Cl. 2 per cent.; 2 cubic centimeters of a 5 per cent. solution of ferrocyanid and  $H_2O$ ) into the peritoneal cavity. Killing the dog in fifteen minutes, I found 130 cubic centimeters in the peritoneal cavity, while the same dog dead a few minutes absorbed 25 cubic centimeters in fifteen minutes. I found that dogs and rabbits just dead absorbed fluid almost as fast as they did while living, and also quite a similar standard prevailed in the same dog, dead or alive. It may be noted that the anterior efferent system of lymphatic vessels pass toward the posterior surface of the xiphoid cartilage and eventually along the mammary vessels, to the



retro-sternal glands, while the two posterior trunks of the central tendon empty into the thoracic duct just anterior to, or above, the point where it emerges from the diaphragm. The lymphatic vessels of each half of the diaphragm can communicate with each other. The lymph trunks of both systems are characterized by possessing spindle-shaped endothelia, many valves and by lying chiefly between the two tendinous layers and the pleural serosa. The lymphatic capillaries emptying into these lymph trunks are characterized by sinuous endothelial walls which possess no valves and have very irregular excavations in different localities and directions. Now, since we have a circular layer and a radiating layer of tendon bundles in the centrum tendineum, we will have straight or radiating lymph capillaries in the intertendinous space of the radiating bundles of tendons. Also straight or circular lymphatic capillaries between the circular tendon bundles. We may call them together intertendinous lymphatic spaces or capillaries. These have quite a uniform breadth, with lateral sinuses or bulgings at various intervals. We also have another kind of lymphatic capillaries which lie chiefly under the pleural serosa and are of irregular and variable breadths. Hence we have two kinds of lymph capillaries. 1. The intertendinous, which run straight in a radiating direction and in a circular direction corresponding to the directions of the tendinous bundles of the centrum tendineum. These two systems of deep and superficial lymphatic capillaries communicate generally where they cross each other by short straight vessels; sometimes a large lymph vessel will suddenly become narrowed and pass between two tendon bundles to gain the other side of the tendinous layer. 2. The lymph capillaries which lie mainly under the pleural serosa, of variable breadth, with sinuous endothelia and provided with excavations.

Peritoneal injections holding coloring matter will demonstrate the various kinds of lymph capillaries in the centrum tendineum. The mechanism of the diaphragm is wonderfully constructed for the purpose of acting like a sieve, as a great and rapid absorbent. The chief aid to this absorptive capacity is the motion given to the centrum tendineum by respiration. The narrowing and widening of the tendinous bundles, due to contraction and expansion of the diaphragmatic muscle in respiration, urges onward the lymph current. The motion of respiration acts on the centrum tendineum like a pump. The deep and superficial straight lymphatic capillaries aid especially in forcing onward the lymph stream through the spreading and closing of the intertendinous bundles. Again, as persistent anatomic exactness is not characteristic of the lymphatic system, we find peculiar deviations, spiral lymphatics, outside of the various systems mentioned.

Whatever may be the functions of the superficial straight lymphatics

of the centrum tendineum, they absorb a wonderfully large amount of coloring matter from the peritoneal cavity in a very short time, both in the living and dead animal. So far as my experiments are concerned, no place in the centrum tendineum is so active in absorption as are the superficial, straight or radiating lymphatics. It appears that respir-



FIG. 94.—(Author.) Drawn from woman's omentum majus. Age 30 years. (Oc. 4, obj. 3, R.) Showing trabeculae absorption or atrophy of inter-trabecular spaces and a few endothelial cells. 1, 2, 3, endothelia covering trabecula; 4, 5, 6, intertrabecular spaces. Note that the atrophy is where the blood vessels are limited. It shows how the artery is divided and assumes a course in the middle of the trabecula. A few blood corpuscles are noted in the artery. 7, 8, 9, trabeculae bared of endothelia. The animals which I have examined showing the most trabeculated condition of the omentum are: 1st, the horse; 2d, the cat; 3d, man; 4th, the dog. Then follow others which are not so definite.



FIG. 95.—(After Handbook for Phys. Lab., Vol. II., 1873.) A fat tract from the omentum of an injected guinea-pig. (a) Artery. (b) Vein. (c) Dense system of capillary vessels of true fatty tissue. (Oc. 2, obj. 2.)

ation dilates and contracts the radiating intertendinous spaces to a very marked degree. The straight, deep and superficial lymphatics seem to me to be the very important lymph channels of the centrum tendineum. In respiration the straight lymphatics are widely dilated, while in expiration they are narrowly contracted, thus allowing them to fill and empty

during the motion of inspiration and expiration. The straight, deep and superficial lymphatics connect the anterior and posterior systems of lymph vessels of the centrum tendineum, becoming in this manner an important factor in the onward progress of the lymph through the sieve-like tendon. Perhaps in this respiratory motion lies its evolutionary process, its gradual steps toward its present condition in mammalian life. With the acquisition of more and more lung tissue the diaphragm assumed a wider range of activity. The wider range of diaphragmatic activity brought with it a necessity of considerable separation of musculo-tendinous bundles at the maximum point of diaphragmatic expansion, because the origin and insertion of the diaphragmatic muscles, musculo-tendinous bands are very various and arranged in an irregular circle, i. e., on the irregular costo-vertebral internal circumference. As all tissue spaces are the recipients of variable quantities of lymph, the intertendinous spaces of the centrum tendineum from their periodic narrowing and widening not only become lymph receptacles but important channels for lymph currents, whose extent and rapidity of flow rests on the regularity of diaphragmatic activity. Ages on ages of mammalian life with increasing pulmonary tissue and power only tend to fix and increase this peculiar but significant function of the centrum tendineum. Again, during expiration the large liver presses upward on the musculo-tendinous bundles of the diaphragm, tending to increase the intertendinous spaces and enhancing the lymph flow. An examination of a rabbit or other animal's diaphragm will quickly reveal the vast intertendinous spaces and the enormous amount of fluid they may contain when the tendon bundles are widely separated from each other, and how very much fluid would necessarily be forced out by their tendon bundles approaching each other and closing up the vast spaces. One can not assert, perhaps, that all the tendinous bundles are separated during expiration, now that all approach each other during inspiration, but that in all probability the musculo-tendinous bundles separate and approach each other once during one respiration, i. e., a complete act of inspiration and expiration. Therefore, we may assert that the chief absorptive power of the centrum tendineum is due to its straight, deep and superficial lymphatic spaces and the rapidity of the absorption is due to respiratory movements. It must not be overlooked, however, that the quiet diaphragm of a just killed animal will absorb almost as fast as the living one. Death (in non-septic cases) probably does not alter the diaphragmatic structure for four to six hours. In one case I found Berlin blue particles absorbed in the human diaphragm seventy-two hours after death. The experimental physiology of the centrum tendineum will be reported in detail in another place, but in some thirty carefully planned and executed experiments we learned some



salient physiologic features of the tendon, which may be summarized at the end of the chapter.

The first matter of surprise with every experimenter in the absorptive capacity of the peritoneum is that the chief point of active peritoneal absorption is the serosa of the diaphragm of man and other mammals. The discoverer of this significant anatomical and physiological fact was Dr. Daniel Recklinghausen in 1862, while experimenting on the peritoneum of rabbits. He demonstrated the matter by the use of Ag. NO<sub>3</sub> and by injections into the lymph spaces under low pressure. Later Recklinghausen showed that the lymph vessels of the diaphragm were profoundly influenced in puerperal peritonitis.

The best method by which to solve the question of the power of the diaphragm to absorb is to inject the peritoneal cavity of a rabbit with Berlin blue suspended in fluid and then to kill the rabbit from a few minutes to 24 hours afterwards. The diaphragm should be cut out and

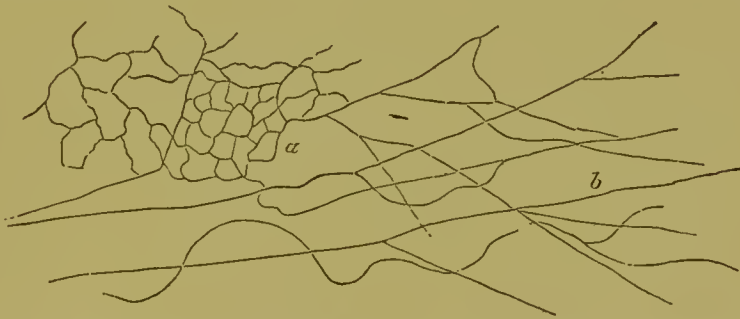


FIG. 96.—(After Handbook for Phys. Lab., Vol. II., 1873.) Network of elastic fibres from the fresh mesentery of a rabbit, treated with dilute acetic acid. In (a) the network is more superficial than in (b). (Oc. 3, obj. 7.) At (a) the elastic network resembles endothelia in outlines. The rabbit's mesentery contains a vast amount of elastic fibres:

treated by various methods before examining it with the microscope. The method to test the normal absorbing power of the human diaphragm is to remove it as soon as possible after death and then place it on various kinds of fluid, putting it to a microscopical test afterwards. To test the power of absorption of the normal diaphragm of rabbits, I experimented chiefly with Na.Cl. 1 per cent. solution in Berlin blue (sufficiently for coloring), alcohol just sufficient to keep the Berlin blue in suspension. In an ordinary rabbit we injected into the peritoneum some four to six ounces at blood heat. The animals were killed 10 minutes to 18 hours later. It may be stated that the coloring matter should not produce a diffuse color, i. e., it should not be a soluble coloring material, but innumerable fine grains should be held in suspension in the fluid so that the grains will be carried into the subserous tissue and be deposited. Diffuse coloring matter would color all the adjacent tissue and obscure structures. After microscopic experimen-

tations on the rabbit's peritoneum with the alcoholic solution of Berlin blue, one finds that the colored granules of Berlin blue have passed from the abdominal cavity to the subserous lymph channels in the diaphragm. The process by which the particles of coloring matter pass from the peritoneum into the lymph spaces is through the stomata vera which are so numerous on the peritoneal surface of the diaphragm, or by local expansion of the interendothelial space. The stomata connect the diaphragmatic peritoneal surface with the subjacent lymph channels. The stomata are vertical channels, lined by granular germinal endothelium, one end of which terminates in the peritoneal cavity and the other end in the subperitoneal lymph space. To experiment on a human diaphragm it should be cut out of a fresh cadaver with as little trauma as possible, then suspended by threads so that its abdominal surface is a depression or concave. Then any kind of fluid desired can be poured on, as milk, alcoholic solutions of Berlin blue or carmine. Another plan is to place the diaphragm over a funnel with the abdominal side downwards, then fill the funnel with enough of the desired fluid to allow the diaphragm to come in contact with it. The colored fluid poured on the diaphragm or allowed to remain in contact with it should be tested at varying intervals, say from ten minutes to two or more hours. In the rabbit the whole thickness of the diaphragm can be viewed through the microscope, while the diaphragm in man is so thick that it must be split and one can shave off a piece of the surface with a razor. In quite a number of examinations of human diaphragms microscopically, I have so far never observed any difference between the diaphragm of man and mammals. However, most of my labors were with the rabbit, dog and frog. I think Bizzozero states somewhere that the diaphragm of man and mammals is similar. Oedmansson states practically the same. It is suggested by Radjewsky, who labored under Recklinghausen's direction in his Strasburg laboratory, that a thin layer of the fluid for absorption should be poured on the human diaphragm so as to avoid any weight. Simply pour a thin layer over the abdominal surface of the peritoneum and leave it for 3 to 24 hours, after which wash it carefully in distilled water. Small pieces from its surface can be snipped off and examined in a drop of glycerine, or it can be hardened with alcohol or Muller's fluid or osmic acid. Experiments on animals vary greatly in regard to results obtained, which must be chiefly owing to the trauma the diaphragm suffers during the experiment. The significant structures of the diaphragm are its lymph channels and stomata vera. Its significant function is its absorptive power inducing a fluid stream toward it in the abdominal cavity.

#### THE LYMPHATICS OF THE DIAPHRAGM.

The diaphragm is a vast bed of lymphatic vessels and spaces. Von

Recklinghausen was the first to show the importance of the lymphatic system in the diaphragm. Ludwig, Schweigger-Seidel and Klein perfected his labors. The method used to observe the lymph vessels was to kill a rabbit, pencil the diaphragm in situ on both thoracic and abdominal sides with a toothpick on which was wound a little cotton. The brushing should be done very gently, brushing the surface three to five times. The cotton brush should be wet in the serum of the peritoneal cavity. The animal should be carefully opened that the diaphragmatic surface may not be smeared with blood. Now pour over both abdominal and pleural surfaces of the diaphragm Ag.NO<sub>3</sub> solution of  $\frac{1}{4}$



FIG. 97.—(After Klein) represents the peritoneum covering the central tendon of the diaphragm of a rabbit. It is stained with silver nitrate. It shows well the stomata vera: s and s show open stomata, while two of the stomata are closed or collapsed. The stomata are surrounded by germinal epithelium; t shows the tendon of the diaphragm covered with irregular shaped and sized endothelium; l is a lymph channel, which we recognized by the small, sinuous endothelium covering it. The stomata vera, or the stomata surrounded by germinating epithelium, are found most on the diaphragmatic tendon, while, so far as my own examinations are concerned, the pseudo-stomata can be found abundantly in the mesentery of the cat. Note that the stomata vera are directly in communication with the underlying lymph channel. 1, The opening and closing of these mouths no doubt have much to do with allowing infection to get into the lymph channels.

per cent. strength and allow it to remain in contact with the diaphragmatic surfaces 3 to 10 minutes; then with a sharp pair of scissors carefully cut out the tendinous portion of the diaphragm, with the least possible trauma, and place it in a vessel containing distilled water. For immediate examination snip off small pieces of the thinnest portion and mount in a drop of glycerine. If the experiment is carefully performed one observes a beautiful network of large lymphatic vessels, especially on the pleural surface, and frequently on the peritoneal. At first failures are liable to occur because one does not know just how to brush away the endothelia. The lymphatics are known by the posses-



sion of numerous valves and by their large size. One can preserve the specimen mounted in glycerine for a long time by renewing the glycerine or hermetically sealing the edges of the cover glass with melted paraffin. For future use the diaphragm may be preserved in alcohol or 5 per cent. formalin. One can observe vast areas of capillary lymphatics and channels on the peritoneal surface of carefully prepared specimens. To inject the diaphragmatic lymphatics, take an alcohol solution of Berlin blue suspended in a 1 per cent. solution of Na.Cl. solution and inject a half-ounce into a rabbit's peritoneal cavity at blood heat. Kill the rabbit 10 minutes to 20 hours later and the lymphatics of the diaphragm will be found loaded with granules of Berlin blue. The colored granules will be observed to pass chiefly into the lymphatics in rows between the tendons of the diaphragm. The diaphragmatic lymphatics are divided into trunks and capillaries. The trunks or main branches have valves while the capillaries do not possess valves, and it is by the test of valves that the vessels are distinguished. The typical place of all the peritoneum to observe the lymphatic vessels is the pleural surface of the diaphragm. Sometimes one can note that the lymphatic trunks narrow down like the point of a horn and dip downward, but this probably forms a narrow connection with some other trunk; or, perhaps its lumen narrows to pass between the tendons of the diaphragm to reach a lower plane. The vessels on the diaphragm are all intimately connected, constituting a vast system, though they are in several layers. If the specimen be carefully brushed and silvered one can observe on the interendothelial space which separates the endothelia of the vessel wall dots and rings, stomata and stigmata around which so much contention has existed for a quarter of a century. Into the great lymphatic trunks possessing valves pour the vast bed of capillary lymphatics, which we easily recognize by their sinuous endothelia. The lymphatic capillaries possess in the diaphragm lateral bulgings or excavations which are very large in parts. It is easy to recognize two kinds of capillaries in the diaphragm. One kind lies between the tendinous bundles of the diaphragm while the other lies immediately subperitoneal or subpleural. The capillaries lying between the tendinous bundles of the diaphragm are straight, possess lateral bulgings or excavations and are covered with a peritoneal endothelia more sinuous and irregular than that covering the adjacent tendon.

The lymph vessels of the diaphragm form meshes, so far as I can observe, the same as in man or rabbit, directly under the endothelial surface. They seem perfectly analogous in all mammals. The lymph capillaries and blood capillaries differ so much from each other that they will not be confounded, for the lymph capillaries are much wider than the blood capillaries. If some small lymph capillaries do resemble

blood capillaries, the lymph capillary can soon be distinguished by following it up to a big trunk, where the valves may be observed and the varicose swelling of the lymph vessel which is so characteristic of the lymph vessels. The diaphragm shows meshes, quite crooked lymph trunks, which may cross each other at right angles at which point may exist a kind of lymph reservoir. The lymph canals in the diaphragm present many apparent variations, for they run between the tendinous bundles of the diaphragm which consist of at least two layers. Now, these lymphatic systems belonging to each layer of tendinous bundles



FIG. 98.—(Author.) Drawn from Ag. NO<sub>3</sub>, stained omentum of boy, not pencilled. Omentum of boy, two years old, (oc. 2, ob. 8a. R.) on a trabecula. Carefully following the specimen. Main trabecula sketched. 1, 1, stomata with granules in the center. 2, nucleus with nucleolus. 3, endothelia. It shows well the various directions of planes of fibrous, i. e., reticulated fibrous tissue.



FIG. 99.—(Author.) The pleural surface of the diaphragm of a woman, 26 years old. (Oc. 2, obj. 8a, R.) It shows three stomata vera, 1, 2 and 3, closed. 1 is long and oval-shaped. 2 is doubtless closed, yet it is clear in the center, and perhaps it did not stain beyond the very dark outer ring. 3 is a very plain, very distinct, closed stoma verum, showing three granular cells. In this single microscopic field are seen a varied appearance of stomata vera. 4, 5 and 6, common pleural endothelia of the diaphragm. The dots on the endothelia appear to represent projections or cilia. Stained by Ag. NO<sub>3</sub>.

connect with each other by short vertical canals where they cross each other. The lymph canals between the bundles are very various, as are also their lateral bulgings. In experimental labors we found quite a difference between the colored granules filling the intertendinous lymphatics of the diaphragm and the lymph capillaries of the serosa

not directly over or between the tendinous portion. The reason is that the meshes of the capillaries between the tendon bundles are larger than at other points. The mesh-work of capillaries in the diaphragm differs considerably in size at different points. The lymph channels of the diaphragm are so numerous and vast that an alcoholic solution of Berlin blue being injected into the peritoneal cavity, in a few hours will make the whole diaphragm a dark blue by filling the lymph spaces. As the Berlin blue remains longer in contact with the diaphragm it produces more irritation (inflammation) and the lymphatic vessels dilate, the colored granules extend wider into the channels and the color deepens, demonstrating that the diaphragm is a vast bed of lymph channels. Of considerable interest is the lateral bulgings or lateral excavations of the lymph capillaries which are found in the centrum tendineum. They generally branch off at right angles and may extend club-shaped or triangular shaped in the lateral direction. Our own experience on rabbits' diaphragms with Berlin blue demonstrated that the fissures or splits between the tendinous bundles of the diaphragm were canals bounded by endothelia, were lymph capillaries enclosed by a nucleated endothelial membrane. Radjewsky demonstrated the same fact by the "puncture method," i. e., by injecting colored fluid with a fine pointed syringe directly into the intertendinous space under the diaphragmatic serosa and afterward examining by the microscope. He found that the colored granules followed exactly the space which the physiologic peritoneal injection did.

It is the diaphragmatic intertendinous straight lymphatic capillaries which manifest the pronounced absorption and deposition of particles of Berlin blue. The two systems or the various systems of lymphatics are connected in the diaphragm by short, straight lymphatics. Klein suggests that they act as pumps between the two systems. Muscatello demonstrated that 5 to 7 minutes after colored granules were injected into the peritoneal cavity they will be found in the diaphragmatic lymphatics.

In general in the physiologic method of injection of the diaphragm, both the peritoneal and pleural sides are filled with the colored granules. But occasionally one side will show filling, and these erratic or discrepant actions of the lymph vessels of the diaphragm I do not fully understand, for the experiments seem to be performed under fairly similar conditions. Of course, the only explanation at hand is unequal effect of technique. A curious feature not only of the diaphragm but of the whole peritoneum is the rapid accumulation and the rapid disappearance of fat. Fat lobules are simply connective tissue corpuscles distended. Fat globules may be observed in the diaphragm lying in the meshes of the lymph vessels. The fat globules accumulate along the



blood vessels and make the structures non-transparent, and it is observed that fat globules and lymphatic vessels are intimate relatives and exist in close association. In fact, some authors regard fat tissue as a kind of gland tissue. Radjewsky showed by experiment that the diaphragm would absorb much more in a state of inflammation than in a normal condition. In the diaphragm of man and mammals I have frequently examined closely under the serosa on either peritoneal or pleural side, where one can observe peculiar stellate formed objects, granular, almost identical with a connective tissue corpuscle. They are doubtless lymphatic canals, for in these spaces Berlin blue or carmine collects. These stellate, irregular lymph spaces are similar to the irregular spaces found in the intertendinous bundles of the diaphragm.



FIG. 100.—(Handbook for Phys. Lab., 1873.) Peritoneal surface of centrum tendineum of rabbit, colored with silver, showing the lymph capillaries of the abdominal serous covering in the region of the large blood vessels which pass through the diaphragm. The sinuosity of the lymph endothelia is typically representative.

Under low power the stellate shaped spaces appear filled with a granular fluid. However, all experimental observers agree that the diaphragm has the special function of absorbing finely divided colored granules suspended in fluid. My own experiments demonstrate that other portions of the peritoneum have very little power to absorb coloring matter. Dubar and Remy have demonstrated that other points of the peritoneum than the diaphragm will absorb colored matter. Others have confirmed the work. Radjewsky, working under Von Recklinghausen's directions, has shown that the diaphragm is a special location of absorption, and he drew some excellent cuts to illustrate his views. It had long been suspected that the lymph and blood vessels had a very close

connection, and when Aeby, Auerbach and Eberth showed that the structure of blood and lymph vessels was similar, it heightened the belief in the experiments of Recklinghausen on the diaphragm and brought out many new observations. But before this Lessing and Fuhrer believed the lymph and blood vessels were connected, as they termed it, by plasmatic canals. Maffucci by experimental investigation showed a dozen years ago that there were several other places where absorption would occur in the peritoneum.

#### STOMATA.

It is on the diaphragm of man and animals, and especially on the mesogaster and mesentery of frogs, that all investigators observe the so-called stomata. The absolute typical stoma is found on the lymph sacs of frogs. So one observes on a well silvered specimen of a rabbit's diaphragm through the microscope there may be certain peculiar structures which may be much more intensely granular than the adjacent endothelia. The structure often consists of two or more intensely colored endothelial cells which surround an opening. There may be 3 to 5 granular endothelia surrounding the opening (stomata). One can observe a distinct oval nucleus in the cell. Klein called these intensely granular polyhedral cells which surround the stomata germinal endothelia. The peculiar cells which form the stomata are very irregular in number and distribution. Sometimes it appears that there is a single granular or germinal endothelium standing isolated and alone, but by careful examination it may be seen that this apparently singular granular endothelium is composed of several granular cells surrounding a small central opening, the real stomata. The stomata surrounded by the granular endothelia are very irregular in shape and size in the diaphragm, which is their typical locality. Sometimes one can only distinguish the cells surrounding the stomata by these granular conditions, for their size and shape do not differ, distinctively, from the adjacent common endothelia. It is better for the amateur to begin with the frog's mesogaster, where the granular polyhedral cells surrounding the stomata are very distinct, and so peculiar in shape and distribution that no possible mistake will long continue with the observer. The stomata on the *cysterna lymphatica magna* of frogs are, I am convinced, of a little different nature than those of the normal peritoneum. The stomata on the frog's lymph sacs have regularity of distribution and number, are constantly present and of definite anatomic structure, and can always be easily stained with  $\text{Ag}.\text{NO}_3$ . These might first be carefully observed. But the frog's mesogaster or less certainly the mesentery should be first studied for reliable knowledge to use in subsequent study in the increasing complexity of the peritoneum of the ascending animal scale. The stomata of the diaphragm are of significant impor-

tance on account of the results of experiments, viz.: that the diaphragm is the only territory of the peritoneum possessed of pronounced absorptive power for solid particles. And all experimenters since Von Recklinghausen (1862) have recognized the phenomenal rapidity with which the centrum tendineum will absorb finely divided granules. The human diaphragm does the same, as I have cut it out 15 hours after death and placed it in Berlin blue, and it will show a blue color from absorption in a few hours.



FIG. 101.—(a) (Author.) Abdominal side of diaphragm. Dog's (three months old) diaphragm. Cent. tend. Abdominal side. Ag.  $\text{NO}_3$ . (Oc. 2, ob. 8a, R.) 7, 7, membranalimitans from which the endothelia have been shed; 1, stomata vera; 2, 2, 2, stomata spuria, interendothelial stoma.



FIG. 102.—(b) (Author.) Pleural side of diaphragm. Drawn from pleural surface of three-months-old dog. (Ag.  $\text{NO}_3$ .) 1, 1, 1, stomata vera; 2, 2, 2, stomata spuria; 3, 3, 3, nucleus. This, no doubt, covers lymph vessels.

The centrum tendineum or zona tendinea should be the portion examined. Though the peritoneum covering the diaphragmatic muscularis shows absorptive powers, it does not compare in capacity to the centrum tendineum. The blood vessels of the diaphragm form a rich plexus. The blood vessels are comparatively numerous, as are the lymphatics. They are derived chiefly from the diaphragmatic and intercostal vessels. The blood and lymph vessels are in close and intimate relation, as may be observed by the results of peritoneal injections in the enormous and unnumbered emigration of the leucocytes. Almost as soon as a foreign fluid is injected into the peritoneal cavity the centrum tendineum begins to be swarmed with leucocytes to battle against invaders. Whatever force induces a condition where the leucocytes wander out of the blood and lymph vessels into the peritoneum appears to increase their numbers, or perhaps it induces the leucocytes to congregate locally at points of irritation.

There are other localities of the peritoneum which furnish typical examples of certain structures such as the omenta, the mesenteries, and the tunica vaginalis. But we have mentioned them more or less in various portions of the book perhaps sufficiently. The frog's mesentery



shows well the stomata vera and typically the blood vessels coursing through lymph sinuses. The blood vessels are invaginated by the large lymph sinus. In other words, the blood runs through wide lymph spaces. Immediately under the peritoneum of the pylorus are wide and extensive lymph channels. The shape and size of the endothelia, the size and number of lymph channels and the stomata vary greatly at different localities of the peritoneum, and it would increase the size of this volume too much to enter into characteristic details in regard to the various localities.

#### THE LYMPH NODES.

We have previously observed that the lymph apparatus of the peritoneum consists of three parts, viz.: (a) the lymph vessels and capillaries; (b) the lymph nodes and (c) the thoracic duct. We have considered the lymph vessels and capillaries of the peritoneum as much in detail as the scope of the work will allow. We will now consider very briefly the lymph nodes of the peritoneum. The lymph nodes, conglobate glands or glands of the peritoneum are very numerous. For example, the mesenteric nodes or glands are estimated to be 250 alone and those of the meso-colon at 50. This simply indicates that they are very numerous when those connected with the whole peritoneum are estimated.

The lymphatic vessels and capillaries of the peritoneum empty into the lacteals, so that the lacteals collect the lymph fluid from part of the peritoneum and act just as the lymph trunks do. The sizes of the peritoneal lymph nodes are from points just visible to the naked eye up to that of a small plum. The shape is round, oval, irregular, bean or kidney-shaped, which represents the chief form. The peritoneal lymph nodes are very widely distributed. In the mesenterium they are arranged in a distinct series. They cluster along large and small blood channels and especially about the lumbar region. Late researches show that though some lacteal vessels reach the thoracic duct without passing through lymph node, most of the mesenteric lacteals are interrupted by several lymph nodes. The lacteals or lymphatics before entering the bean-shaped nodule divide into several branches (from 2 to 6). The entering lymphatic vessel is known as the vasa inferentia. The lymphatic vessels leave the gland by a few divisions which immediately after exit from the node unite into one common trunk. The vessels leaving the glands are known as vasa efferentia. The lymph gland is covered with a capsule composed of connective tissue and sometimes muscle. The capsule penetrates into the gland forming septa which conduct and distribute blood vessels to the various regions. The depression in the gland at the point

where the capsule dips into it and where the vasa efferentia pass from it is the hilum. The cortical portion of the gland is composed of the follicles, and the medullary portions is the darker, spongy reticulum. Each lymph gland is enclosed in a fibrous covering of varying thickness. The covering is composed of connective tissue cells, with fibrous and elastic tissue. Nerves, blood vessels and occasionally muscle fibres occur in the capsule. The outer portion of the glandular covering merges gradually into wide-meshed connective tissue cells and fat cells. The capsule of the lymph node on its internal surface gives a wide system of



FIG. 103.—(Author.) Omentum of horse, perhaps twelve years old. 1, 1, stomata vera; 2, 2, nuclei; 3, rift in cell edge; 4, stoma verum cell with two nuclei; A, stomata vera with two cells, each having a nucleus (note it has nine cells around it); 6, an extension of the stain; 4, stoma verum, five nuclei in it; 7, a granular cell of stomata 1 (Oc. 4, ob. 3 R.: there are here four stomata vera very granular); 11, rifts between cells; 12, stoma spurium; 5 shows stomata vera indefinitely divided with a nucleus to each cell. This drawing is of epithelia lying adjacent to regions with innumerable stomata vera. It is from the surface of a trabecula. The horse's peritoneal endothelia is characteristic for peculiar irregularity and grouping of endothelia. This endothelium lies in a germinal tract.

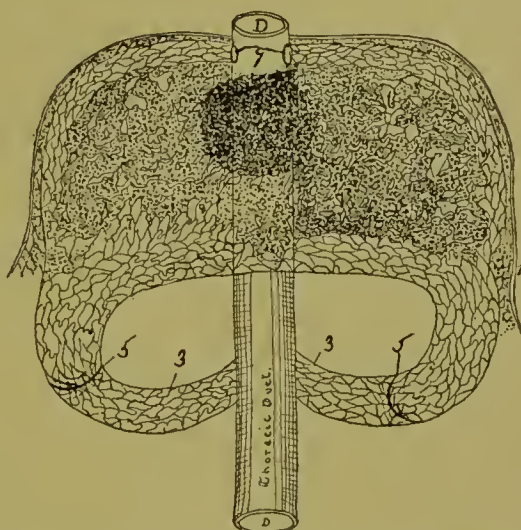


FIG. 104.—(Author.) Diagram to illustrate the lymphatic drainage of the centrum tendineum of the diaphragm into the two anterior and two posterior trunks. D, D, upper and lower end of the thoracic duct; 1, outer end of the diaphragm; 2, anterior lymph trunks passing on the posterior surface of the xyphoid appendix to accompany the mammary arteries; 3, 3, two posterior trunks of lymphatic vessels which drain the centrum tendineum and empty into the thoracic duct; 5, 5, valves of the trunks; 6, 7, 8, are dilated lymph spaces in the tendon. The black spot represents the point of the centrum tendineum where the heart rests on it, at which point there are no lymph spaces; 9 represents the mouths of the two anterior channels.

partitions or septa. The partitions divide the node into many and various sized departments which contain the lymphoid tissue. The septa are of the same composition as the capsule, i.e., made up of fibrous connective and elastic tissue with perhaps some muscle cells. His observes that the septa of the lymph nodes of the mesenteric glands of the ox contain considerable muscular tissue. The septa are perforated so that the various compartments of the gland communicate with each other. The partitions of fibrous tissue divide the gland into various

roundish bodies which are known histologically as the follicles. The follicles do not come in contact with the septa. Between the follicles and capsules there is a space called the "investing space of the follicles." The follicles vary in size, number and arrangement in the same animal, and also in different species. The size of the gland depends on its physiologic condition.

The elastic fibres allow it to contract and expand to accommodate itself to activity and rest. Through this complicated structure, the lymph node, the lymph fluid has to pass. What modifications arise in the lymph as it passes through the mesh-work of the gland is not all made known. Not only fluids pass the lymph node, but solid particles. It may be here noted that the typical subject in which to observe the mesenteric lymphatic glands is a six-months' foetus or an infant up to year old, but both should be as spare as possible. The infants dying of long-continued and wasting disease are the typical subjects for observation. However, one is never so forcibly impressed in regard to the peritoneal lymph glands as when he sees them enormously enlarged by disease as in tuberculosis. The peculiar reticular frame-work is the special feature of lymph node. It is the reticular frame-work of adenoid tissue. From these considerations we observe the lymph glands are composed of a system of cavities which communicate with each other. The cavities contain lymphoid material. The cortical portion of the gland contains the lymph follicles while the interior portion known as the medullary portion contains the lymph tubes. A peculiarity of lymph glands is that the lymph follicles do not come in contact with the fibrous system of septa, but is separated from them by what is known as "investing space." Neither do the lymph tubes come in contact with the septa, but lie separately on "intercommunicating passages." To learn the course of the lymph through the nodes we must resort to artificial injection. Frey was the first to determine the course of the lymph through a node in 1860. His soon afterward ascertained the same fact. Hyrtl's "puncture" method has been rich in results, especially by Teichmann in his work on the lymphatic system published in 1867 and dedicated to Hyrtl. Frey, Hyrtl, Teichmann and His have shown by artificial injection that the fluid first enters the gland and finds its way into the investing spaces of the follicles. The investing spaces of the node are in connection with the inter-communicating passages of the medullary tubes. It is seen then that the vasa efferentia must take its origin from the passages about the medullary tubes.

The nerve supply of the peritoneal lymph node is imperfectly known. We know that the blood vessels carry nerves wherever they pass, and the nodes have a rich supply of blood vessels. Koelliker traced nerves into the glands. Up to date the literature in regard to the nerve end-



ings of the peritoneal lymph nodes is very scanty. Lymph nodes are all constructed on a similar type, which consists of reticular tissue modified by vascular supply. The methods of preparation of lymph nodes will be given in the chapter on technique.

Autopsies and clinical experience demonstrate that the lymph nodes of the peritoneum are localities of high physiologic activity. We observe this pathologic fact in tuberculosis and typhoid fever. In infants of less than a year old in certain forms of disease is found a most typical enlargement of the mesenteric lymph nodes.

#### ADIPOSE TISSUE.

In the omenta of all animals examined was found a deposit of fat cells which must be considered as a form of subperitoneal tissue.

In general fat cells are connective tissue cells expanded with oil.



FIG. 105.—(Author.) Pleural surface of the diaphragm of a woman, 26 years old, (obj. 8a, oc. 2, R.). It represents one very beautiful stomata verum with granular, polyhedral cells. The stomata is open. 1, 2, 3, 4, common surface endothelia grouped around the stomata. Highly stained with Ag. NO<sub>3</sub>.



FIG. 106.—(Toldt) represents the endothelium from the great omentum of a child six weeks old.

Toldt and Klein consider fat cells to belong to a peculiar glandular group. Under the microscope it appears to be composed of beautiful round globules which are transparent. Fat cells are supplied by a rich network of blood vessels. The connective tissue network generally divides into bundles which form what is known as lobules. Each lobule has its own blood supply. The elements of developed fat tissue are the cells, closely aggregated spherical cells. It can be seen under the microscope that the covering of a fat globule is a thin membrane which contains in its wall at some point the nucleus. The fat tissue is distributed along blood vessels sometimes in an ornamental style, fringing the

blood vessels. In young growing animals it may be observed in more or less elegant streaks in the omentum of a variable color. The rise and fall of the fat globule is a peculiar feature. At first small, fine globules collect in the connective tissue cells; later the globules become confluent and form one or two spherical bodies enclosed in a fine mantle. In the disappearance of the fat the oil globule assumed the appearance of fluid or liquid, and the liquid gradually absorbs, when the expanded tissue corpuscle becomes again the collapsed one. Doubtless the nerve endings of the said expanded connective tissue cell are damaged, for the collapsed fat cell seems to lose its old function as in mobile kidney. In serous membranes the fat tissue is derived from a peculiar multiplying connective tissue, from nodules and cords. As the matrix of the nodules increase there arises a reticulum or network resembling adenoid tissue. Fat tissue or lymphatic tissue cannot be separated in their development; they are congeners. Nodules, tracts, cords and fat tissue develop side by side and so far as can be observed, it seems impossible to differentiate between that which will be ultimately fat tissue and that which becomes lymph tissue. These lymph nodules multiply lymph cells. But as the cells in the lymph nodule become converted into fat cells they seem to reproduce new lymphoid cells. Here then we have a structure, as Klein remarks, functioning as a lymph gland at one time and at another as fat tissue.

The full details as to the minute changes which occur when a protoplasmic connective tissue cell is transformed into a fat cell, with its differentiation from lymph tissue, is more extensive than the scope of this work includes. The reader is referred to the more special labors of Flemming, Klein and Toldt.

It is noted that there is a peculiar yellow tint of coloring matter which belongs to the fat cells and varies slightly in each species. The fat of rodents and ruminants is quite hard in consistence. That of man and carnivora is much softer. Ether and hot alcohol will dissolve out the oily part of the cell, leaving a flaccid, empty connective tissue cell. The physiologic utility of fat cell or tissue in the peritoneum is questionable. It can act as pads or buffers, but are such needed? It may aid in distribution of pressure, but it increases intra-abdominal pressure and thus aids in producing hernia. It may aid in retaining heat. We are not in full possession of the knowledge of how the fat cells are produced, but doubtless the protoplasm of the cell body is much concerned. In the embryos of about the fifth week the fat cells accumulate along the blood vessels of the omentum and mesenteries. The period of fat deposit in the peritoneum of animals is quite variable. In the fat cells of the peritoneum under certain conditions the oily contents become crystalline. The crystals are fine, needle-like objects.

The quantity of fat cells associated with the peritoneum is frequently enormous, especially connected with the omentum, ligamentum peritonei and kidney. We have seen the omentum an inch thick and almost burying out of sight every abdominal viscus. The size of the fat globule is generally from 1-300 to 1-600 of an inch in diameter.

## FAT.

The appearance and disappearance of fat is a peculiarity of the peritoneum. The utility of the fat in the peritoneum is not fully understood. If the peritoneal fat suddenly disappears this may produce ptosis of the



FIG. 107.—(Author.) Young dog's lymph vessel found on gastro-splenic omentum. 1, 1, cells of lymph canalicular system in direct connection and continuation of the endothelia of the lymph vessels as it is with lymph capillaries. Now this vessel is not as well or clearly marked in outline as some are. It is, however, a lymph space or sinus. 2 represents a space of germinating endothelia which is not differentiated into distinct endothelia. Many stomata spuria and a few stomata vera exist. This might be looked on as a lymph capillary.

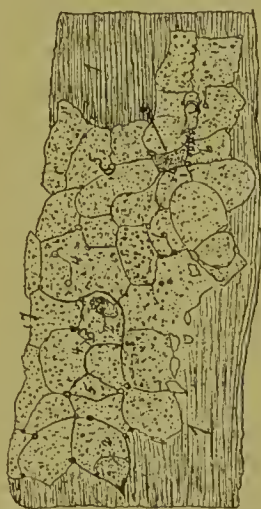


FIG. 108.—(Author) Drawn from a band of peritoneal adhesions which stretched from one part of the omentum to another, in a man 48 years old. It was a typical white cicatricial inflammatory band. The Ag.  $\text{NO}_3$ ,  $\frac{1}{2}$  per cent. staining was done 30 hours after death. 1, sub-erosus tissue or ground substance. 2, 2, nuclei; 3, an endothelial cell thrown off; 4, 4, intraendothelial stomata; 5, 5, stomata vera; 6, stomata spuria; 7, free edge of trabecula. This is a very significant figure as it shows that peritoneal exudates can act like the old peritoneum, i. e., the exudates become covered with peritoneal endothelium.

kidney. It becomes very movable, especially upward and downward; the ligamentum peritonei elongate and their insertions appear to shift downward or canalward. The stretching of the peritoneum by fat lobes seems to destroy its elasticity to a certain degree. The disappearance of usual bobbins of fat from the hernial orifices predisposes to hernia from widening of the hernial orifice. A fat, stiff omentum cannot become rolled up. The appendicæ epiploicæ, after being once widely distended with fat, do not seem to return to the original size. The utility of fat in the peritoneum as a store-house may be great to hibernating animals



which have long periods of fasting, but to man fat in the peritoneum can be looked on as a detriment if in considerable quantities. It seriously increases intra-abdominal pressure, endangering hernial processes. Doubtless a thick, fat-filled omentum majus may keep the viscera from hernia and volvulus, and may aid in preventing the spread of pus. To attribute to the fat in the peritoneum the power of keeping the body warm is a poor excuse for such a waste of tissue. While fat is packed about organs they seldom suffer dislocation (inter-optosis), but when a considerable quantity of fat suddenly disappears it predisposes to visceral prolapse or ptosis (entero-ptosis). The young of animals have less fat in the peritoneum than adults. Young children have little fat in the omentum except along the course of its vessels.

Knowledge of the utility of fat in the peritoneum might be obtained by comparing the amount that certain species of animals possess while living on different foods. The peritoneal fat arises about the sixth or seventh month of foetal life and is chiefly deposited along the large blood vessels first, and later it becomes general over the surface. The fat globules develop among the trabeculae of connective tissue.

In the peritoneum there can be but little doubt that the fat cells develop from lymphatic tissue; probably the lymphoid cells become developed into fat cells. Some histologists believe that adipose tissue and fat tissue are closely related. Others, however, disclaim any relation by noting that when the fat cells disappear the cells again resemble connective tissue and not lymph cells.

#### PIGMENT CELLS OF THE PERITONEUM.

Situated immediately underneath the peritoneum, especially of the frog, may be seen some of the most beautiful pigment cells. But as they are merely large connective tissue cells loaded with darkly colored pigment, it will not concern us sufficiently to study them in detail.

In concluding some views on the subserous tissue, we must remember that it is difficult to discriminate always the original connective tissue cells from forms which seem to be a modification of it or a deviation from it. We have learned that there is a vast system of connective tissue cells, or branched cells forming the matrix of the subserous tissue. We have noted that where these branched cells are well supplied with blood they multiply and proliferate, producing large tracts. We observed that the lymph fluid flowed between the branched cells bathing all the subperitoneal tissue with a nutrient and draining fluid. This fluid is the life of the branched or connective cell; it brings to it nourishment, it floats away debris or bits of protoplasm, it produces a medium to exercise its function

of expansion and contraction. The connective tissue cells are so fastened in an elastic net that they are capable of varying to a vast degree to accommodate the varying volumes of lymph fluid which flows between them. It appears that the connective tissue cell is the father of the lymphoid cell, and also that it is transformed into an endothelial cell as it juts up between the edges of the cover-plates. It is also the father of the fat cells.

The transformation of a connective tissue cell into an endothelial cell appears to depend on its reaching the surface. [We note that when connective tissue cells are well supplied, the blood vessels not only mean rich food, but much oxygen.] We observe the accumulation of lymphoid cells in an extensive manner. It must then be considered that these lymphoid cells may be converted into connective tissue cells, fat cells, endothelial cells of three varieties, viz.: those which line the peritoneal cavity, those which line the blood vascular system, those which



FIG. 109.—(Author.) The peritoneal side of the diaphragm of a woman, 26 years old. Dead three days. Ag.  $\text{NO}_3$ . (Oc. 4, ob. 3.) The figure shows four stomata vera, 1, 2, 3, 4. Enormous numbers of stomata vera exist on the peritoneal side of the diaphragm, more than were found on the pleural side.

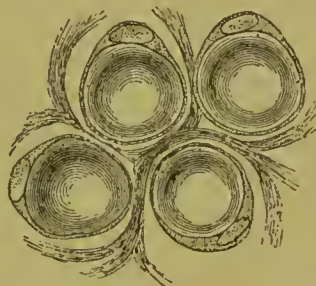


FIG. 110.—(After Handbook for Phys. Lab., Vol. II., 1873.) Ordinary fat cells of a fat tract in the omentum of a rat. (Oc. 3, obj. 7.) Observe the nucleus in the connective tissue capsule at the side. The connective tissue cell has expanded widely and covers the fat cell or globule like a mantle.

line the lymph vascular system. We must view the complicated process of nourishment and growth as yet imperfectly understood. In the vast, panoramic germinating tracts of the peritoneum, both endothelial and branched cells characterize the vigorous, active points of growth, or proliferation. The proliferation occurs by budding division. The lymphoid corpuscles may be considered the key to health and disease. They lie and live in the lymph fluid, the nourishing fluid of all fluid, and these prepare themselves for future life. Like the individuals of a nation, they gradually become differentiated into laborers of a definite variety. It is when this formative protoplasm becomes confused in its progress, diverted from its final object, that wild growths and disease arise. It seems as long as the panoramic scene of growth which occurs in the omentum, for example, definitely confines itself to the limits of its own cells no life process is disturbed, but as soon as the irregular growth-processes strike other parts, as the fixed, branched

cell, the process of disease begins. As a matter of fact, the various scenes of growth of both endothelial and subendothelial character cannot be absolutely placed in a field of growth or disease. We cannot always discriminate one from the other, so near do they blend and border the one on the other. As in the vegetable organism, life and death follow each other very closely.

#### THE GROUND SUBSTANCE OF THE PERITONEUM.

In this subdivision we will consider the ground substance peculiar in its formation, structure and function to the peritoneum. Certain cellular elements are elsewhere considered. My investigation of the ground substance of the peritoneum has been carried on almost entirely on the omentum majus of animals. Perhaps a rabbit's omentum will serve the best purpose if it be spare and free from fat. There is considerable difference and variation among the omenta of different animals and even in the same species of animal at different ages. Old animals have more opaque patches than the young. But rapidly developing and luxuriant ground cell substance is best seen in young animals; especially good for this work is the young dog.

It may be remembered that the omentum is a panorama of growth and change. The small opaque patches of the young animal coalesce in the older animal to form opaque areas. By staining the omentum with  $\frac{1}{4}$  per cent. of  $\text{Ag. NO}_3$  in situ, we can observe the differently situated patches turn a rich brown color. Sometimes the color is of a very rich brown. These patches are covered with germinal endothelia. But the patches and tracts really belong to the ground substance. One must pursue a systematic method to observe any distinct results. The plan followed after several trials was to bleed a rabbit by severing the carotids, open the body immediately and carefully pencil or brush the omentum majus on one side with a toothpick wound with cotton, wet with peritoneal serum. Brush three to five times quite lightly. Now pour on the brushed omentum  $\frac{1}{4}$  per cent.  $\text{Ag. NO}_3$  and allow it to remain from three to six minutes, when the portion of the omentum to be examined is cut out with a sharp pair of scissors and conveyed to a capsule of distilled water. If left a couple of hours in the capsule with sunlight it will show brown spots here and there. One can then carefully examine the various spots by mounting small bits in glycerine.

Until one has had considerable experience in brushing or pencilling the omentum frequent failures are apt to arise. If the ground substance be brushed too much, its cellular relations are destroyed and we view simply confused bundles and granular masses not unlike jelly. The endothelia may not be brushed sufficiently to remove them, and hence is observed the cellular elements of the granular substance. In well prepared portions of omentum with the endothelia brushed off, we may



note the groundwork of a small portion. We see a beautiful network of branched connective tissue cells forming a typical mesh-work or reticulum. The branches of the cell are in distinct and typical connection with the capillary blood vessels, showing that the blood vessel wall and the branched connective tissue cell is one and the same thing. The branched connective tissue cells are flat, and between their anastomotic branches may be observed the juice canals of Von Recklinghausen. The flat

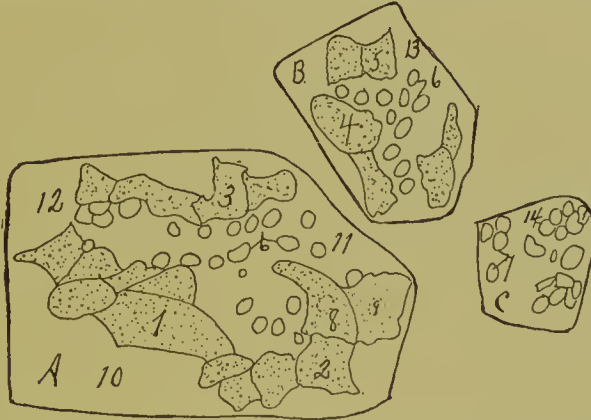


FIG. 111.—(Author.) Drawn from the abdominal side of the diaphragm of a girl 13 years old to illustrate the pores in the membrana limitans. This diaphragm was removed 72 hours after death. It was stained with Ag. NO<sub>3</sub>  $\frac{1}{4}$  per cent for 10 minutes. It was then placed in carmine colored fluid for 10 hours, where it absorbed considerable numbers of colored granules of earmine. It was then placed in Muller's fluid two days, sections prepared by snipping off small bits of the zona tendinea and mounted in glycerine. The endothelia of the diaphragm were in general mostly desquamated, and especially the cover-plate and its edges were much dissolved. In this diaphragm the apertures of the membrana limitans appear mainly in groups. The groups are in general 10, 20, 30 and 40 in a group. Some groups of apertures in the membrana limitans appear to be 75 or 100, yet this may be where two or more groups coalesce. The size of the holes would appear large enough to admit from 1 to 4 red blood corpuscles abreast. Single holes may appear alone in the membrana. There is no doubt that some of the bright spots in the cover-plate represent the apertures in the membrana limitans, as it is plain to see in this diaphragm. Some have interpreted the bright spots in the cover-plate as holes peculiar to it. The holes are round, oval, oblong, square, but chiefly round and oval. The maceration of the endothelia and their falling off by merely washing and rinsing is one of the best ways to observe the holes or pores. However, on vast areas of the diaphragm one cannot see the pores. Hard brushing destroys distinct views of the pores. 1, 2, 3, 4, 5, endothelia isolated and connected; 6, 6 and 7 show the shape, size and relation of the apertures in the membrana limitans; 8, 9, nuclei of endothelia; 10, 11 and 12 in A and B and 14 in C, show the membrana limitans with its pores. It resembles a soap bubble in filling, but is visibly granular.

anastomosing connective tissue cells form a firm mesh-work which is very adjustable. The net- or mesh-work appears elastic and can be observed under the microscope surging backward and forward in any fluid current which may exist. It is evident that Recklinghausen's juice canals occupy a large space in these branched connective tissue cells. The cells lie in the lymph canicular system surrounded and bathed by the lymph, and it is surprising how much space these canals occupy in a majority of the ground substance of the omentum. Now, in this mesh-

work of connective tissue cells certain brown elements may be observed. The elements have a nucleus and often one or more nucleoli. These brown elements have the characteristic migratory cells. They are larger than white blood corpuscles. Also one observes elements which correspond to lymphoid corpuscles. The characteristic of branched cells or of the germinal cells of the ground substance is their flatness. They are really flat granular plates. If we move the specimen in the microscopic fluid until we come to a place in the ground substance of the omentum which has a rich supply of blood capillaries, we will observe that the chief matrix consists of granular branched nucleated cells whose processes are in direct connection with the walls of the blood vessel. Coursing through the mesh-work which the branches of the cells form are the juice canals of Von Recklinghausen. It depends on the amount of lymph in the tissue how wide the lymph canals present. The branched connective tissue cells may be closely crowded together or the clear spaces or juice canals may be quite wide. The ground substance shows well "fixed" cells, and beautifully after the specimen has lain in Muller's fluid for several days. The number of branches which the cell presents varies greatly. Some are numerous, long and fine, while others are short, thick and few. To make any classification of the new growth of the ground substance of the peritoneum is difficult and perhaps clears up the subject but little in the reader's mind. However, some division may be easily made.

In the mesentery of a frog, we find a beautiful network of germinal endothelia by staining with Ag.  $\text{NO}_3$ . It resembles a mosaic. This network of germinal endothelia rests on germinating cells of the ground substance also. The germinal endothelia are smaller than the common surface endothelia. The germinal endothelia exist in two special forms. One form consists of cones or knobs which are raised above the level of the common endothelia and are of a darker stain and more granular than the adjacent endothelia. The cone has a pedicle which is much narrower than its club-shaped head. It varies in its elevation above the common surface and is only on one side of the omentum. The knob of germinal endothelia may be of any shape, and it indicates its localized origin from a stoma verum or spurium. The germinal endothelia of this knob-shaped mass rests on granular cells of the ground substance, and the cells of the ground substance seem to have the power to produce endothelia to cover them as they project upward above the common ground substance. Again, one sees a kind of net- or patch-work of germinal cells of the ground substance all definitely covered by darkly granular germinating endothelia. They are raised above the general or common surface. We then have as distinct classification of the ground substance:

1. An accumulation of germinal endothelia which covers accumulating cells of the ground substance.

2. We have an accumulation of flat granular, nucleated branched cells. In the flat branched cells may be observed the lymph canalicular system which, according to the condition of the tissue, will show wider or narrower spaces, i. e., according to the amount of lymph fluid the tissue contains.

3. We have what Klein has so beautifully pictured in his cuts very vascularized patches. These one can very nicely observe by hardening in Muller's fluid after brushing off the endothelia. But I secured the best specimens to show vascularized patches in the ground substance of the omentum by first brushing off the endothelia and second, hardening in Muller's fluid 48 or more hours, and third, coloring in acid fuchsin



FIG. 112.—(Author.) Drawn from the pleural side of same diaphragm as Fig. 109 (woman 26 years old). The figure shows three stomata vera, 1, 2, 3. No. 2 is wide open and Nos. 1 and 3 are closed, while No. 1 shows a slight mouth and No. 3 nuclei for two granular cells. No. 2 has six granular cells around its mouth. The stomata vera here (pleural side) are larger than in Fig. 109 (peritoneal side). 4, a granular cell or a cell much more browned than others.

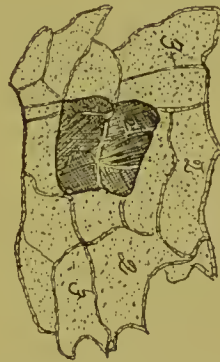


FIG. 113.—(Author.) Hen's diaphragmatic serosa. (Oc. 4, obj. 8a.) 1, stomata verum closed with six granular, polyhedral cells surrounding its mouth; 2, 2, common endothelial cells; 3, 3, double interendothelial lines. This stomata verum, with a large number of other stomata vera, lie in a distinct row between the tendons of the diaphragm.

for over two hours. One can then observe the vessels connected with the branched cells of the ground substance quite accurately. The vessels and the branched cells form a network and the juice canals pass in every direction bathing the tissue in lymph.

4. Finally, we have nodules of the ground substance which resemble adenoid tissue. The matter of adenoid tissue in the peritoneal ground substance is a subject which has impressed many observers.

The following is from the excellent labors of Klein: "We will, however, anticipate by calling the patches and tracts which we have been considering up to the present lymphangial patches (nodules) and lymphangial tracts. We may therefore say that there exist in the omentum of the rabbit two kinds of lymphangial structures.



a. Patches, the matrix of which consists of ordinary, more or less flattened, more or less branched cells which on the one hand multiply by division, in which way the patches increase in size and from which on the other hand grow up lymphoid cells. The branched cells lie in the lymph canalicular system together with lymphoid cells. At an early stage of development, these patches do not contain a special system of blood vessels; at a later turn they possess a special rich system of mostly capillary blood vessels. By growing in length these patches join so as to form whole tracts.

b. Patches and tracts, the matrix of which consists of a reticulum, the meshes of which contain a variable number of lymphoid corpuscles. They are generally provided with more or less abundant blood vessels." To observe these structures we should study omenta which are sufficiently thin to be examined by the microscope as they are found in the animal. The main conception of the cellular elements of the ground substance of the omentum (peritoneum) is that it consists of flattened, branched, nucleated protoplasmic cells, and the lymph canalicular system of Recklinghausen represents the space in the ground substance of these cells. It may also be noted that the protoplasmic cells of the ground substance play a significant role in the panoramic growth-changes of the normal omentum, and these cells play a still more important change in chronic peritonitis. The omentum is a very important peritoneal appendage. It is a capillary drain to the peritoneal cavity, directing, no doubt, the peritoneal currents. It is a man-of-war ready at a moment's notice to sail to parts invaded by infection. It prevents visceral adhesions to the anterior mobile wall. It is the chief peritoneal protector against invasion. The very reason it is so important to the existence of a perfect peritoneum is because it is so frequently found crippled and fixed, maimed in the early defense of checking an infectious enemy. The omentum is a guard to protect the peritoneal interests and a sentinel to warn off infectious intruders. But as it is the first volunteer to check peritoneal invasion, it is also one of the first protectors to become injured by fixation. Though the lymphatics will be separately discussed, we find in the cellular elements of the ground substance peculiar forms of growth which E. Klein has designated by the term endo-lymphangial and peri-lymphangial. The term peri-lymphangial refers to the development of the cellular elements around a developing lymph space. The nodules and tracts develop around and lie outside the lymph vessel. The peri-lymphangial nodules or tracts have a significant relation to fat areas. We must look for peri-lymphangial tracts along blood vessels, and of course we look for fat areas along blood vessels, for it is characteristic to note the elegant streaks of fat in the omenta of all mammals along blood vessels. Fat tracts and peri-lym-

phangial tracts stand in directly opposite relations. The more fat the fewer peri-lymphangial tracts, and the more peri-lymphangial tracts the less fat tracts. In this statement there is a significant relation between cellular elements which will develop into fat or lymph spaces. Toldt, Flemming and Klein appear to think that fat is a kind of glandular element, or at least a close relative of it.

In young dogs and cats the fat tracts predominate, but in rabbits with an almost fatless omentum, lymphangial tracts and nodules appear considerable. From a microscopical study of quite a number of omenta of animals of different ages I found that the lymphangial and fat tracts were quite variable. Klein states that the lymphangial tracts and fat tracts in the monkey and guinea-pig are about equal; but on these ani-



FIG. 114.—(Author.) Horse's omentum to show endothelia grouping around a stoma verum. 1, 2, 3, endothelia; 4, rift between cells (the endothelia appear to be new themselves; they are surrounded by long fields of new or germinating endothelia); 6, 6, show some of the adjacent growing endothelia; 7, stoma verum (oc. 4, ob. 3); 8, 8, show a field non-prepared which lies on the border of still newer germinating endothelia (6, 6); 9, rift between cells, i. e., a shrinkage of the granular protoplasm.

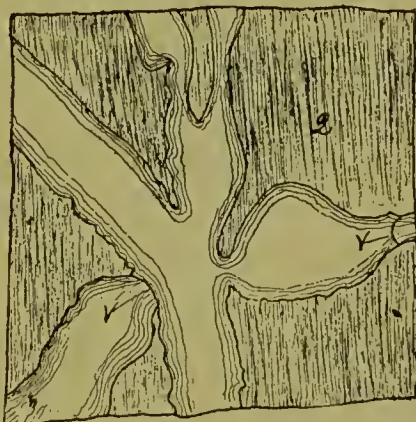


FIG. 115.—(Author.) Lymph vessels of pleural serosa of diaphragm of rabbit. (Ob. 4, oc. 3, R.) The surface was well pencilled and then stained with a  $\frac{1}{2}$  per cent. solution of  $\text{Ag. NO}_3$ . v, v, valves. Note the irregularity of outline. Ground substance dark.

mals my study has been limited on account of lack of material. Klein's observation is that in the mesentery the lymphangial nodules or tracts have the greatest tendency to develop into fat tracts, and this agrees with others.

Some of the best published articles on the subperitoneal tissue are those of the English authors, Makins and Anderson. The literature of this subject, however, is quite scarce. The subperitoneal tissue is of almost equal import to that of the peritoneum in surgery. But in no portion of the body is the knowledge of anatomy more necessary to understand the diseased processes than it is in the subperitoneal tissue.

The subperitoneal tissue, vast, divisible shiny planes of mesoblastic tissue are primarily developed about blood vessels. It is a widely distributed complicated structure, chiefly existing on the dorsal wall where

the great blood vessels first appeared. In the embryo laterally it gradually spreads with the growth of the visceral laminae. The subperitoneal tissue passes outward with the vascular subdivisions to the viscera, padding well the course of the blood vessels.

It is in this subperitoneal connective tissue, elastic tissue, nerves, blood and lymph vessels, all firmly interwoven into a web which constitutes the *membrana mesenterii propria*, and which is faced on one or both (mesenterial) sides by an endothelial membrane—the peritoneum proper. In the mesentery the endothelial layer of both sides may be stripped or peeled off the *membrana mesenterii propria*, i. e., the essential neuro-vascular visceral pedicle. The subperitoneal tissue is arranged chiefly in planes which glisten and shine like the peritoneum itself, in fact, may be easily mistaken for the peritoneum. The flat, thin, subperitoneal planes which can be split and resplit contain fat and are traversed in various localities with unstripped muscular fibres, as the muscular fibres in the broad ligament and the muscle of Treitz or the *musculus suspensorius duodeni* (mesenterium) serve as good examples, but they may be found in the mesentery and mesocolon.

In many dead subjects one can split and resplit the planes of subperitoneal tissue to an almost unlimited extent. The interstitial spaces, of course, are the lymph spaces.

In some regions the subperitoneal tissue is vast in extent, as the dorsal region along the *psoas* muscle, around the kidney at the base of the broad ligament and in the prevesical space or *cavum Retzii*. The amount of subperitoneal tissue varies not only in different individuals but in the same individual at different periods of life.

The contents of the subperitoneal tissue are very varied, but chiefly consist of the great blood vessels around which it primarily developed. It contains the sympathetic nerves with their ganglia, i. e., those between the pelvic and thoracic diaphragm, the *receptaculum chili* with its ducts, the ureters, lymphatic glands, the lumbar and sacral plexuses of nerves. All the above-named structures are covered by sheaths of subperitoneal tissue. The subperitoneal tissue contains the relics of the foetal life, as the *Wolffian* body, the *parovarium* and *Müller's* duct.

The unstripped fibres act as supports extending from the fascia lining the abdomen to the viscera. *Treitz's* muscle is the best example. It is a wide fan-shaped muscle with coarse strands of unstripped muscles united by much connective tissue. It is perhaps a relic of some ancient animal life, for in the amphibia we may observe considerable unstripped muscular fibres in the subperitoneal tissue. The relations of the subperitoneal tissue are that it lies between the abdominal parietes and peritoneum in some places and in others between the viscera and peritoneum. It lies on the *transversalis*, *diaphragmatic*, *iliac* and



pelvic fasciae as a continuous sheet around the abdomino-pelvic wall. The sheet is the thickest about the kidneys, at the base of the broad ligament and along the psoas muscles, and thinnest where it is closely fixed to the abdominal walls, as the linea alba. In its peritoneal relations it is loosely attached to the



FIG. 116.—(Author.) A diagrammatic profile view of the lymphatic duct with its visceral tributaries and nodules and two posterior lymph trunks from the diaphragm. 1, internal jugular vein; 2, innominate vein; 3, subclavian vein; 4, thoracic duct; 5, 5, 5, 5, the two anterior lymph trunks which drain the anterior portion of the diaphragm and accompany the internal mammary vessels; 6, 6, the two posterior lymph trunks which drain the posterior portion of the diaphragm emptying into the thoracic duct; 7, 7, a portion of intestine to illustrate the lymph capillary vessels; 9, 9, lymph capillaries; 10, 10, 10, the mesenteric lymph nodes; 11, receptaculum chyle; 12, lumbar lymph nodes or glands; 13, diaphragm. This figure represents the three great divisions of the lymph system: a, the non-valved variable lymph capillaries (9, 9), with sinuous endothelia and excavations emptying into the lymph trunks (10, 10) and nodes; b, the valved lymph trunks with the intervening lymph nodes emptying into the thoracic duct; and c, the thoracic duct (3) emptying into the subclavian vein.

wall of the peritoneum. It is this loose attachment of the peritoneum to the subperitoneal tissue which endows the peritoneum with such capacity to shift on its base and allows operators to strip the peritoneum from organs and parietal

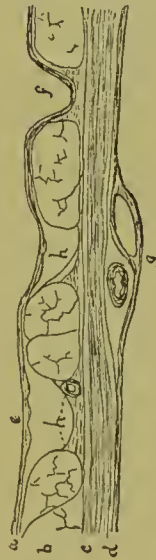


FIG. 117.—(Author.) (Ludwig and Schweigger-Seidel, 1866), is a section of the centrum tendineum of a rabbit. 150 diameters. a, the peritoneum, d in the pleura; b represents four of the radiating tendon bundles; h, h, shows the tendinous lymph spaces, one fully and the other partially distended; f illustrates the peritoneum over a lymph space between the tendon bundles, depressions, i. e., the fluid lymph has disappeared from the channel; g is a blood vessel; e is the circular fibrous layer; c represents the peritoneum put on a stretch over the inter-tendinous lymph space.

walls for peritoneal cuffs and grafts to repair wounds and cover denuded viscera. The loose connection of the peritoneum allows extra-peritoneal operations on abdominal viscera by separating the peritoneum from its subperitoneal bed, as in kidney operations, colotomy, extra-peritoneal ligature of the iliac vessels, Alexander-Adams' operation and operations on urachal cysts. In short, the shifting, loose bed of subperitoneal

tissue allows innumerable operations in plastic peritoneal surgery. The subperitoneal tissue holds a very variable relation with the viscera. The kidney is surrounded entirely by a thick bed of subperitoneal tissue as well as the renal vessels and ureters and supra-renal capsules.

Foetal relics are also well buried in it. The subperitoneal tissue forms Glisson's capsule and extends into and over the liver. It is a very thin sheet between the liver and peritoneum. It forms a complete sheath for retro-peritoneal organs, as the pancreas, vessels, nerves and ducts (ureters, Wolffian and thoracic ducts). In its relation to the mesenterial supports it passes from the abdominal parietes to the viscera forming the real neuro-vascular visceral pedicle, the *membrana mesenterii propria*.

It forms such a shifting bed that the vertical colons are accommodated within a wide range of expansion and contraction. It forms the well-known connective tissue bed on each side of the cervix which has been the fierce battle-ground for gynaecologists for a generation. It forms a vast bed lying on the recto-vesical fascia which covers the levator ani muscle.

The subperitoneal tissue is prolonged extra-abdominally on all the nerves and blood vessels which pass out of the abdomen in various directions. The vessels and nerves are ensheathed in this tissue and run with them in all directions. The subperitoneal sheaths, as Mackin and Anderson assert, generally contain four compartments, viz.: one for an artery, one for a vein, one for a lymphatic and one for a nerve. We may summarize the subperitoneal tissue by saying: It surrounds and ensheathes the retro-peritoneal structure. It ensheathes every vessel and nerve and accompanies them as they pass from the abdomen. It lies between the blades of visceral folds and it lies in contact with all the abdominal organs. A careful consideration of its anatomical structure will lead the way to a better comprehension of the extensive pathology which may be found in the subperitoneal tissue. For example, an ischio-rectal fossa may induce a subperitoneal abscess in the pelvis by the infection following the sheath of the haemorrhoidal vessels and nerves, and vice-versa.

#### CONCLUSIONS.

1. The diaphragm is the characteristic muscle of mammalian life, beginning imperfectly in crocodiles and birds.
2. The centrum tendineum consists of four layers, two of serosa, abdominal and pleural, and two layers of tendinous bundles, the radiating and circular.
3. The characteristics of the serosa are stomata vera and spuria and the interendothelial space. The interendothelial space is divisible into two parallel lines and crossed by anastomotic protoplasmic pro-

cesses, resembling a railway with its ties. The endothelial cells are held together in colonies by transverse protoplasmic processes.

4. The characteristic of the tendinous layers is that they possess the deep and superficial straight lymphatics between the bundles, and that these straight lymphatics connect the anterior and posterior lymph system of the diaphragm.

5. The intertendinous lymph spaces between the radiating bundles of the centrum tendineum are covered by a sinuous endothelia of smaller dimensions and more irregular than covers the adjacent bundles of tendons, and possess numerous stomata vera arranged quite regularly in rows.

6. The centrum tendineum is a bed of lymphatics consisting of valved trunks and capillaries of variable width possessing sinuous and spindle-shaped endothelia, lateral excavations and no valves.

7. The rapidity and extent of absorption of the central tendon is due to the widening and narrowing of the intertendinous lymph spaces depending on the motion of respira-

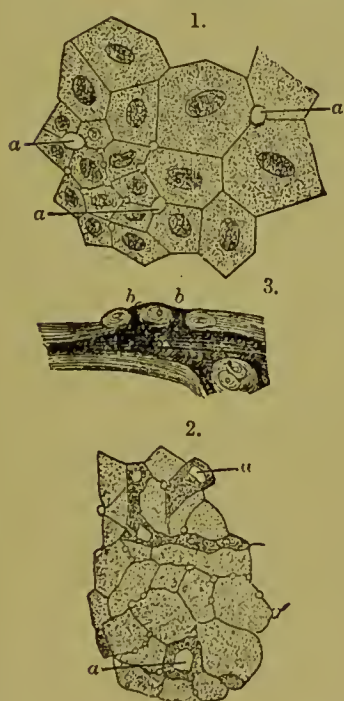


FIG. 118.—(After Ludwig, Schweigger-Seidel and Dybkowsky, 1866-67), are typical figures to represent stomata of the endothelia. 1, nucleated endothelia from the abdominal surface of the rabbit's centrum tendineum. a, a, a, stomata vera. Several others may also be seen at the common junction of several endothelial cells. 2, endothelia of the mesenterium of a dog. a, a, represent typical stomata vera surrounded by protoplasmic granular cells, spreading in various directions in the interendothelial space, i.e., some of the protoplasmic granular cells of the stomata vera have blended. 3, section through the pleura of the dog. b, b, free orifices of short lateral canals of the lymph canals.

tion. In expiration the radiating intertendinous spaces widen and thus span the lymph space between the tendinous bundles. In inspiration the intertendinous spaces narrow, and this forces the fluid onward.

8. The lymphatics of the centrum tendineum stand in open connection with the peritoneum through vertical canals lined by granular polyhedral cells, whose dilations and contractions regulate peritoneal currents.

9. There is a fluid current existing in the peritoneum directed toward the centrum tendineum.

10. The diaphragm or central tendon is the chief place of peritoneal absorption for colored granules. (I formerly thought it the only place of absorption, but experiments show a slight absorption in the



gastro-splenic omentum of colored granules in the region of the pylorus, and perhaps others will be found by search.)

11. The membrana limitans, so far as my researches extended, is perforated only on the centrum tendineum, which gives a physical explanation of rapid absorption of colored granules through the centrum tendineum.

12. The colored granules wander through the central tendon mainly in a free state and to a less extent enclosed in leucocytes.

13. I can not observe any macroscopic, microscopic or physiologic differences between the centrum tendineum of man and other animals.

14. Lymphatics almost fail where the pericardium and central tendon coalesce. Blood vessels are not constant in location in the centrum tendineum; they are mostly found on the pleural, and sometimes on the peritoneal side, often in the intertendinous spaces.

15. The peritoneal serosa absorbs more rapidly than the pleural serosa.

16. The respiratory movements of the diaphragm act like a pump on its lymphatics.

17. Since the dead centrum tendineum absorbs similar to the living, the so-called "vital" process of the endothelial cells must be questioned.

18. It is the lymphatics that play the role of absorptive in the central tendon, and not the blood vessel.

19. The best coloring matter to place in the injected peritoneal fluid to test the physiologic action of the centrum tendineum is Berlin blue.

20. The particles of Berlin blue may be traced in the centrum tendineum in two directions: a, toward the posterior surface of the xiphoid appendix to accompany the mammary arteries; and b, toward the vertebral column to empty into the thoracic duct just above where it emerges from the diaphragm into the thorax. This places the non-valved lymphatic capillaries of variable breadths and possessing excavations in the middle portion of the centrum tendineum, with the valvate lymphatic trunks toward the costal periphery.

21. The vast absorptive capacity of the centrum tendineum depends on its direct connection by means of stomata vera with the peritoneal cavity, on its great extent of lymphatic vessels and spaces on the perforations of the membrana limitans, and on the respiratory motion of the diaphragm.

22. The force of gravity, intra-abdominal pressure and contact of fluids against the centrum tendineum enhances the rapidity of the absorption of colored granules. For if an animal is hung up by the hind legs the absorption is more rapid than if he be made to sit up. Also

movements, manipulation, massage, kneading of the abdomen, etc., hasten the deposit of colored granules into the lymph spaces of the centrum tendineum. So far experiments do not indicate to me that starving the animal hastens absorption to any considerable extent.

23. I can not observe in the experiments that tying one or both thoracic ducts (i. e., the two innominate veins) retards absorption of the colored granules into the centrum tendineum. But the experiments have shown that if the thoracic duct (i. e., the left innominate vein) be tied, the injected peritoneal fluids appear in the bladder about twenty minutes later than if the duct be left untied. The test is made by injecting fluids into the peritoneum containing 2 to 6 per cent. of potassium ferrocyanid, subsequently every five minutes squeezing the urine out of the bladder and adding ferric chlorid, producing a beautiful blue reaction if any potassium ferrocyanid be present. The test is so delicate that the blue reaction will occur at about 1 to 30,000.

24. The vast and active absorptive capacity of the centrum tendineum with the consequent fluid stream directed toward it is a strong argument against peritoneal irrigation, as any fluid in the peritoneal cavity will quickly stream with its contained germs toward the diaphragmatic tendon, the dangerous grounds of peritonitis. I have frequently observed inflammatory products on the peritoneal serosa in pleuritis and pneumonia. Recklinghausen found violent inflammation on the peritoneal serosa of the centrum tendineum in septic puerperal cases.

It does not seem correct to consider the mesenteries or so-called ligamenta peritonea as simply duplicatures of the peritoneum. They have a more profound significance, morphologically. The original mesentery consists at first of a collection of cells on the dorsal region. Blood vessels, the significant portion of any mesentery, soon become apparent in this heap of primitive cells. The vessels gradually increase and branch continually farther forward in the increasing collection of mesenteric cells. The blood vessels gradually deposit around the connective tissue and weave a sheath about them. The peritoneal or endothelial layer is but a thin covering, a single layer of plates, for the solid web of tissue, the mesenterii membrana propria which contains vessels, nerves and lymphatics. The process by which the mesenterii membrana propria, the real neuro-vascular visceral pedicle, acquires a covering, i. e., an endothelial layer of cell plates, is perhaps similar to that of the skin, it is an epidermization. The fact that the real mesentery nerve loses its real pedicle containing its original blood-vessel trunks is sufficient evidence that a mesentery is not a mere peritoneal duplicature. The peritoneum proper may be shifted into many new mesenteries or supports, but the original mesentery composed of primi-

tive cells, blood vessels, nerves and lymphatics all woven into a web and designated the *membrana mesenterii propria* never changes. In the case of the descending colon the original mesentery has coalesced with the left dorsal wall, the endothelial layer of the left face of the mesocolon descends, and the dorsal parietal layer has disappeared by coalescence or gliding out of the left closing dorsal-mesenterial angle.

Again, the fact that the *membrana propria* of the mesenteries always increase with the growing bowel indicates their original and essential relations. In the first heap of cells along the dorsal wall between the gut and wall, the blood vessels begin to deposit about them in proportion to their size and age. The connective tissue is greatest at the dorsal base and gradually decreases until it almost ceases to exist at the margin of the bowel. Foetal development indicates that the mesenteries are independent primordial structures and not merely peritoneal duplicatures.



## CHAPTER V.

### THE BLOOD VESSELS OF THE PERITONEUM.

Men may come and men may go, but I go on forever.—*Tennyson's Brook.*

The old authors believed the peritoneum to be destitute of blood vessels. Later the belief was entertained that the peritoneum possessed blood vessels which carried in health serum only, but when distended by fluid during inflammatory processes, blood also. Finally, microscopic research demonstrated that the peritoneum is highly endowed with a rich supply of blood vessels. The blood vessels of the peritoneum are endowed from various sources, from the thoracic and abdominal aorta, the intercostal, the diaphragmatic, the branches of the coelia axis, the renals, the lumbar, the spermatic and the iliac all contribute to supplying the peritoneum with arterial blood. The veins are more numerous than the arteries. An artery and a vein generally run together. The typical artery consists of: 1. A lining membrane composed of endothelial plates, so arranged, edge to edge, as to produce a closed tube. In frogs and turtles (amphibia) the arteries often lie in large lymph spaces which might be called peri-vascular lymph spaces. But this is quite difficult to see in man's peritoneum. The application of Ag. NO<sub>3</sub> marks the capillary tubes into nucleated areas. The capillary wall is contractile, a very important fact which produces significant conditions of its walls. 2. An outer connective tissue sheath known as the adventitia. 3. A middle muscular layer composed of transversely arranged muscle-cells which in the stage of rest reaches about the entire circumference of the vessel, so that when it contracts the vessel lumen must narrow. 4. Inside the muscular layer is an elastic membrane known as the intima. We shall merely mention some points in regard to the various layers of the artery, but to the endothelial lining we will pay the chief attention. The tissue forms a network which contains many lymph channels. The muscular layer will not detain us long, for in smaller arteries it consists of simply a membrane of transverse muscle-cells which reach around the vessels in a quiet stage. In large vessels there are oblique muscle-cells and even longitudinal bundles. The methods which have presented the most typical muscular membrane in the arteries of the peritoneum in our work have been secured by placing the specimen in Muller's fluid for thirty hours and then coloring with acid

fuchsin for two hours. The elastic portion of the artery is generally spoken of as the fenestrated membrane of Henle, in regard to which we will not devote time nor space. This nucleated endothelial cylinder is called by His an endothelial tube, a periepithelial tube by Auerbach and a cell membrane by Remak. This delicate endothelial membrane is the constant portion of the blood vessel. It is present in all blood vessels, in the finest capillary, the largest vessel and the heart. The capillary consists of this endothelial membrane only.

The layer of the artery which will interest us is the endothelial layer, i. e., the lining membrane of the vascular tube. This endothelial layer is everywhere a single layer of endothelial cells. They are in general elongated spindle-shaped in the direction of the longitudinal axis of the tube. In the case of the blood vascular endothelia, as we did in the peritoneal endothelia, we shall consider the endothelia of the blood vessels of mesoblastic origin, and hence endothelia and notepithelia. The endothelia of the blood vascular system of the peritoneum are considered by all investigators, so far as I am aware, to be naked. They are not endowed with any cilia or mobile protoplasmic processes. They are of mesoblastic origin and should be denominated endothelia. In many places of the peritoneum we may see the blood tubes composed only of the endothelial plates, and it is to this layer of endothelia which our chief interest will be devoted. The blood vessels of the peritoneum course very closely beneath the peritoneal endothelia, and we can observe them in all grades of construction from the artery containing all the various coatings to the simple capillary tube composed of a single endothelial layer. The endothelial layer of the blood vessels when the silver nitrate is applied to it produces a network of dark lines and spaces. The dark lines represent the spaces between the endothelial cells. Nearly all previous authors claim that the dark interendothelial lines represent a kind of cement, semi-solid or fluid substance. But I shall assume at once that the so-called cement substance, fluid or semi-fluid is hypothetical, in short, does not exist. The dark interendothelial line we shall claim is exactly like the peritoneal endothelial line, which is a space and should be called interendothelial space. This space is crossed transversely by anastomotic protoplasmic processes. The endothelial plate of the peritoneal blood vessel is of exceeding fineness. It is often so thin that with strong light one can not see it. The plate possesses an oval or round nucleus situated centrally or excentrically. Both nucleus and ground-plate have a network of fibres. The shape of the endothelial plate of the blood vessels is elongated, spindle-shaped. It has a serrated, sinuous or curved outline. The elongation of the endothelial plate is in the long direction of the axis. Some cells have an oval shape.

The arrangement of the vascular endothelia seems to me is of primordial origin. They group themselves about certain common points, though the grouping is not so manifest as in peritoneal endothelia. The size of the blood vascular endothelia is in general larger than the common peritoneal endothelia. The long diameter exceeds the short diam-

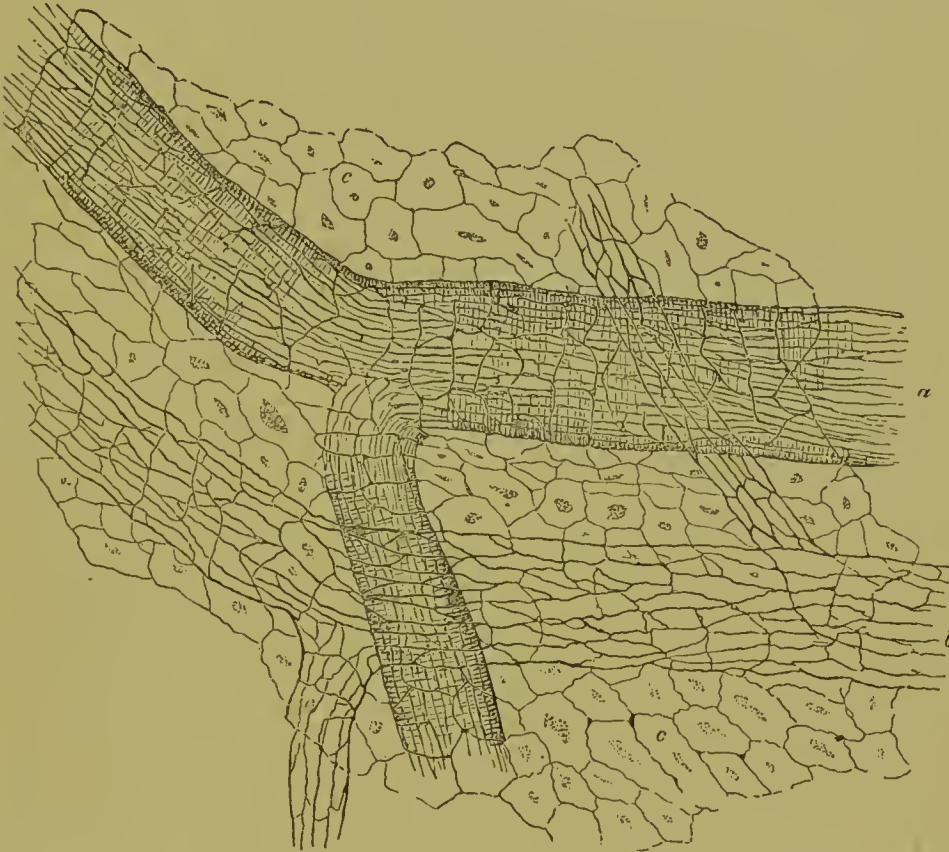


FIG. 119.—(After Handbook for Phys. Lab., Vol. II., 1873.) Omentum of rabbit colored in silver. a, one of the larger arteries, showing the spindle-shaped endothelium and transverse muscular fibre. b, one of the larger veins, showing the endothelial elements, which are not so elongated as in the artery. c, endothelium of one of the surfaces of the membrane. (Oc. 3, obj. 5.) This specimen shows well the transparency of the endothelial membrane exposing the underlying structure.

eter perhaps a dozen times or more. The very elongated endothelia of the blood vascular system is its characteristic. The endothelial plate itself of the blood vascular system is simply a non-perforated thin plate which possesses elasticity only. It is not of any special interest beyond its elasticity, for doubtless fluids do not pass through its walls in ordinary function, so to the physiology of the blood vascular system in its interendothelial space, we will now turn our attention. Our investigations were made on the peritoneum of quite a number of animals. The chief reagents of value are Ag. NO<sub>3</sub>, to darken the interendothelial space; osmic acid or Muller's fluid, to harden and fix structures. Well prepared specimens under a good oil immersion lens show



that the interendothelial space will separate into two parallel lines, one on the edge of each plate, and the space is crossed transversely by fine dark protoplasmic processes. This means that the blood vascular endothelia are organically connected and that the hypothelial cement substance must yield to the term interendothelial space. The blood vascular endothelial plates are identical in every way with the peritoneal endothelial plates, and the interendothelial space of the one is identical with the other. The blood vascular endothelia are not separate independent plates, but represent a cell colony held together by organized, protoplasmic processes, as does the peritoneal endothelia. The blood vascular endothelial plate has an identical structure with the peritoneal endothelial plate. It is composed of 1, a cover-plate; a smooth, hard surface portion, indurated, metamorphized protoplasm. (b). The remaining underlying protoplasmic portion of the endothelial plate contains the nucleus and furnishes the anastomotic protoplasmic process which binds the various endothelia into cell colonies. It is true the anastomotic process which binds the endothelial plates together is not so easy to observe in the interendothelial space of the blood vascular system as it is in the peritoneal interendothelial space for the simple reason that the blood vascular endothelia are exceeding fine, and then of course the processes are more limited in number. There is not, in my opinion, sufficient difference between the blood vascular endothelia and the peritoneal endothelia to attempt to differentiate the one from the other by any distinct characteristic.

The blood vascular endothelia are everywhere flat plates. The plates are thicker in the arteries than in the veins. But it is not easy to decide whether the thickness or the thinness of the plates in arteries and veins is not due to a certain amount of tension or contraction. But our investigations were chiefly carried on by observing the blood capillaries where empty or full blood vessels did not produce manifest differences. On account of the thinness of the plates the interendothelial space is very thin, and hence the network of protoplasmic processes is much more limited and difficult to demonstrate than in the peritoneal endothelial space. It may be here mentioned that Subbotin, it appears a Russian, first declared that the vascular endothelia had no organic connection; later, that if they existed they were so fine as to be invisible and finally, the endothelia rest on each other like tiles on a roof, i.e., the edges overlap each other. I have never seen any such conditions exist as related by Subbotin. We have not even observed a simulation of blood vascular (or any) endothelia even overlapping each other on the edge. Subbotin's pictures must have been owing to some peculiarity of preparation, for I find no other author agreeing with



FIG. 120.—(Author.) An artery drawn from frog's mesentery to show how the emptying and filling of the blood vessel has elongated the endothelium. It shows how adjustable the endothelia are. 1, stoma verum. It appears below the surface endothelia, i. e., its outlines shimmer through the endothelial plate. 2, 2, 2, stomata vera. 3, artery bared of endothelia. 4, endothelia of artery; note how sinuous. 5, 5, 5, endothelia covering lymph vessels. 6, 6, 6, endothelia covering vessel elongated. 7, 7, ends of artery. 8, stoma spurium. 9, intra-endothelial stomata. The endothelia passing transversely across the artery are elongated at the edge of the artery; the endothelia rapidly changes into irregular sinuous endothelia covering peri-vascular lymph spaces.

him. Like the interendothelial space of the peritoneal endothelium, the interendothelial space of the blood vascular endothelia possesses stomata and stigmata or stomata vera and spuria. In the finest capillaries the interendothelial space is very fine and thin, showing short and fine processes. The structures, stomata, which may exist in this narrow interendothelial space must of necessity be exceedingly small. High power and good specimens are required to discriminate structures. As the interendothelial space in the blood vascular endothelia is much more limited than the free interendothelial space of the peritoneal surface, the stomata or stigmata are more difficult to differentiate, and it is in this field that so much polemics arise. Stricker, I think, was the first to demonstrate the permeability of the blood vessel wall for red blood corpuscles, while Waller, Conheim and Hering demonstrated the permeability of the blood vessel wall for leucocytes, however, especially in inflammatory conditions. Yet migrations of white corpuscles (and to some extent red ones) is a physiologic process. It must be remembered that the length of the interendothelial anastomotic processes as well as the interendothelial space undergoes much change according to the pressure in the blood vessel and the circulatory and assimilating disturbances. This change of breadth in the interendothelial space must depend on the contraction of the protoplasmic portion of the cell and the lateral pressure of the blood in the vessel. Also must be considered the non-uniform dilation of the vessel wall. With well prepared specimens of blood vessels one observes black spots or rings in two localities, viz.: (a) at the common junction of several endothelial plates; (b) along an interendothelial line. Some spots are entirely dark, some have clear spaces in the center, others vary in their color. The ones at the common junction of several endothelial plates are irregular in number and distribution. They are very darkly red from the Ag.  $\text{NO}_3$ , no doubt from young protoplasm and considerable precipitable albumen. They are quite irregular in size and shape. However, they are real structures as under all precautions of treatment they arise. These are the stomata vera. Some explain their existence by the retraction of the protoplasm of the adjacent converging cells and plates, others as reproductive centers. Others say they are the result of white blood corpuscles, leucocytes, having passed and left an aperture; others assert that it is merely a local divergence or widening of the interendothelial space where the leucocytes have passed and it is not yet closed. It has been suggested by Goluben, Stricker, Kolossow and Ranvier that the light spots, round or oval, seen in the interendothelial space is an aperture in the membrana limitans. It is suggested that the membrana limitans is perforated or has pores at the place which corresponds to the interendothelial line or space. Kol-



ossow intimates that if it be not the membrana limitans it is the intima which is perforated to correspond to the interendothelial line. This



FIG. 121.—(After Handbook for Phys. Lab., Vol. II., 1873.) a, endothelium of peritoneal surface. b, an arteriole branching into true capillaries d, which are continued into a capillary vein, c. The endothelia is clearly shown in all the vessels. (Oc. 3, obj. 7.) This specimen shows well the blood vessels under the transparent silvered serosa.

would furnish a reasonable explanation of the rapid outwandering of white blood corpuscles or leucocytes in any circulatory or assimilatory

disturbance, for whatever induces the exit of the leucocytes from the blood vessel seems to conduce to their rapid multiplication. Now the last two writers on the subject of stigmata in the blood vessel endothelia, Muscatello, 1895, and Kolossow, 1894, both assert that they are not artificial products, but the results of increased lateral blood pressure and the still enclosed passage of the leucocytes through the interendothelial space. The white spot with the dark ring around it on the interendothelial line may represent a white corpuscle in exit, as the Ag.  $\text{NO}_3$  is able only to precipitate the superficial layer of albuminous fluid.

The stoma verum of the blood vascular endothelia, i.e., the one located at the common junction of several cells, is explained by supposing the retraction of the adjacent endothelial plates has ruptured the organic connection of the protoplasmic or that the basement membrane (intima) has non-uniformly given way. But we must assert that by all precautions we have been able to use, we always found more or less stigmata or stomata present. It may be that the interendothelial space does not expand uniformly under even normal pressure, and hence the spots and dots. The stomata and stigmata are more numerous in inflammatory processes. Of course, one must not forget that the endothelial plate can and does contract and it may not contract uniformly, causing local widenings in the interendothelial space. In these openings, stomata and stigmata, in the interendothelial space of the blood vascular system lies the apparent cause of the wandering out of the white corpuscles or leucocytes. Also the cause of the existence of colored blood corpuscles. Here we might speak of the capacity of leucocytes themselves to pass out of the vessels between the endothelia by their power of locomotion, i. e., by their amoeboid movements. According to Conheim, Binz, Arnold, Lawdowsky, there are conditions of very great venous congestions when the leucocytes do not pass out of the blood vessels between the endothelia. They ascribe this non-exit to a paralyzing of the motor power of the leucocyte by  $\text{CO}_2$ . It is very probable in intense venous congestion excessive  $\text{CO}_2$  exists in the blood. The question would naturally arise, does the leucocyte force itself out of the vessel through the interendothelial space by its own motion, its amoeboid movements, or does it pass out by preformed openings? Does the white blood corpuscle or leucocyte creep out of the vessel by its own inherent power? Does it burrow a hole through the interendothelial space, allowing it to close subsequently slowly or rapidly? Or does the increased lateral blood pressure force the leucocyte out? Lawdowsky, who concerned himself much with the motion of leucocytes, calculated that the leucocyte would travel 1 mm. in about two hours. He calculated this measurement by the comparison of the size of red corpuscles. He calculated that the time required for a leucocyte to pass through a

capillary wall was from eight to forty minutes. A capillary wall is almost immeasurable in fineness, so that the inherent power of mobility of a leucocyte is not only slow, but very slight. Lawdowsky gives a graphic account of how he witnessed a leucocyte meet and penetrate a red corpuscle and pass out on the other side. This he did to show the great capacity of the leucocyte to move and penetrate the interendothelial line. He says the leucocyte met the red blood corpuscle and did not turn



FIG. 122.—(Handbook for Phys. Lab., Vol. II., 1875.) Surface of omentum of rabbit, pencilled and colored in silver. a, lymphatic capillary in the neighborhood of b, an artery. c, capillary blood vessels, the wall of which is evidently in continuity with the numerous branched cell forms, d, in the ground substance. At e, the endothelium of the lymphatic capillary is similarly seen to be in continuity with the cells of the lymph substance. The blood vessel is invaginated at least on one side by lymph vessels. (Oc. 3, obj. 7.)

aside to avoid it, but attacked it on its broadside. The leucocyte contracted itself and projected out a pointed process which began to penetrate the red blood corpuscle. Gradually the process of the leucocyte penetrated through the substance or red corpuscle and passed out of the opposite side, whence it spread out wing-like and drew the remainder of the white corpuscle or leucocyte after it. After the leucocyte had passed directly through the substance of the red blood corpuscle, the canal formed by the leucocyte gradually began to close, and finally the



surface of the red corpuscle became entirely smooth and no trace of the leucocytal penetration was left behind.

Lawdowsky wrote in the Russian tongue, and I am indebted for this account to Dr. Kolossow and the various other translations in the German. However, I must say that I have never seen the leucocyte act so graphically with its indomitable motive power. Kolossow remarks that after two years of observation of movements of leucocytes he has never seen such as described by Lawdowsky. So far as I am able to observe in my experiments, the leucocyte moves in a direction of least resistance, i. e., between fibres and blood corpuscles and not to penetrate through their substance. The leucocyte progressed by movement between the tissue elements and not through them. According to my experiments one can observe, especially on the diaphragm, that the leucocytes come out and go in at definite places and that the places of ingress and egress have peculiar structures and appearances recognized as different from the rest of the diaphragmatic serosa, and, in fact, appearing to be stomata or organized structures whereby the leucocytes pass and repass, i. e., the disturbance of circulation induces the leucocytes to come out and do battle against the invading foe which may be colored granules or other matter and then seize what they are able to carry, and with the diaphragmatic stream pass back to the subserous lymphatics. By careful watching one can sometimes find the leucocyte clamped between the edges of the endothelial plates. Arnold presents some of the most beautiful illustrations (1876) of the various stages of the passage of leucocytes. It appears from my experiments that the leucocyte is possessed of wonderful malleability of plasticity of adjustability to shape. It molds itself in all shapes like fluid jelly. It passes through a much smaller hole than the size of its body. It does this by a kind of flowing of its protoplasm. It first projects out a protoplasmic process to penetrate its way between the elements and finally when a small bit of its process is through it has the power of making the rest of its body flow after it, even through a constricted opening. In fact, if one point of the body can insinuate itself through or between an object, it is capable of drawing the remainder after it and inducing it to flow after it. The scholarly and persistent experiments of Von Recklinghausen demonstrating that pus cells and white blood corpuscles, both having amoeboid movement, have a similar origin instigated vast new researches. The migratory cell was once a leucocyte, and its wanderings from the blood vessel is a physiologic process as well as a method to protect the organism against invasion.

It appears that Waller was the first to observe in the frog with the microscope the passage of white corpuscles through the normal vessels. He attempted to show its important connection to suppuration, but as

Prof. Thoma remarked, it was forgotten until Recklinghausen discovered the wandering cell in connective tissue. Conheim, in 1867, simply revived the emigration theory of the almost-forgotten Waller and announced anew, no doubt, his original discovery of the wandering out of white blood corpuscles from the blood vessels, yet to this day Conheim is credited with the theory by the general profession. As regards the emigration of white blood corpuscles from blood vessels, we must give credit to prominent laborers as J. Arnold, Bottcher, Key, Stricker, Thoma, Metchinkoff, and others, who have extended the knowledge of white corpuscle migration from the vessels. In the emigration of white

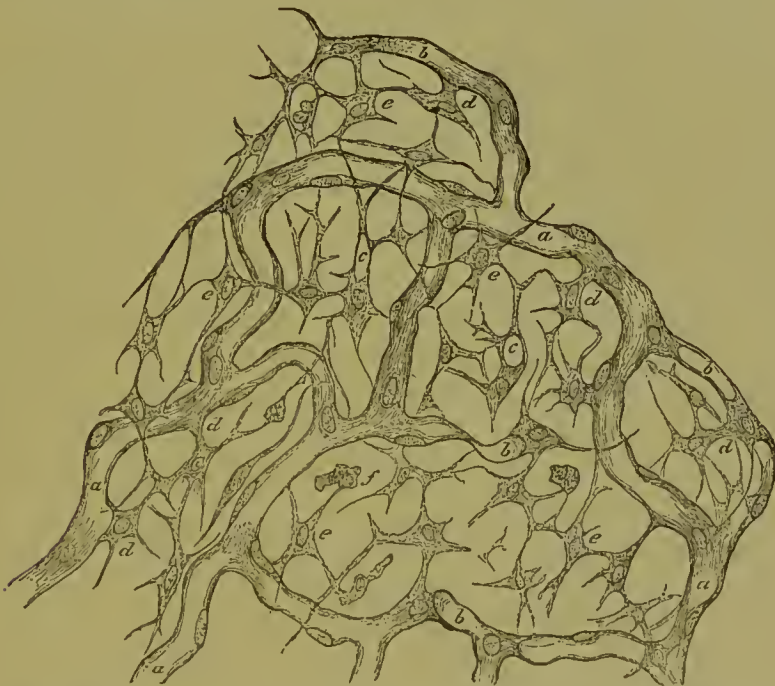


FIG. 123.—(Handbook for Phys. Lab., Vol. II., 1873.) Preparation from the normal omentum of a rabbit, first pencilled and then treated with silver, showing the development of young capillaries. a, capillary blood vessels. b, capillaries only just hollowed out. This process of excavation is taking place in the branched connective tissue cells d, which are in relation with the capillary wall. c, vacuoles in the branched cells. e, branched cells of the ground substance. f, migratory cells. (Oc. 3, obj. 7.) This cut shows excellently the connective tissue cell joining with the walls of the blood vessels.

blood corpuscles from the blood vessels there are many considerations to take into account. It has been shown by the student of Helmholtz and Recklinghausen, Dr. Schklarewsky, that in a glass tube which has fluid with particles of unequal specific gravity in it that the heavier particles collect in the center. Now the red blood corpuscles collect in the middle or axial portion of the blood vessels and the white ones assume a marginal zone, for the specific gravity of red blood corpuscles is greater than that of white. The chief names connected with working out this view are Poisenille, Donders, Gunning and Conheim, i. e., that the red blood corpuscles are chiefly in the central axial stream

and the white ones on the marginal zone. This makes the blood vascular endothelia continually bathed in blood plasma. The specifically lighter leucocyte floats along the wall of endothelia and allows it the first chance of exit. But the leucocytes are apt to be in the marginal position of the blood stream, as it is slower. Thoma says that the labors of J. Arnold and Appert and himself justify the following statements:

1. When the rate of blood flow is rapid there is a margin zone free from blood corpuscles.

2. When the blood stream is moderately slowed the leucocytes accumulate in the marginal zone.

3. When there is very great slowing of the blood stream or when it is quite arrested, the axial current is lost. It appears that slowing of the blood current induces the leucocyte to tend toward the marginal zone. J. Arnold and Thoma, with Engelmann, working under his direction, showed by gradual silver injections in the frog that the white cells of the blood pass out of the vessel between the endothelial cells. The next question to investigate is, what forces draw the leucocyte through the vessel wall? Hering describes it as a filtration of cells through the pores of the vessel wall. Schklarewsky attributed the exit of leucocytes to the axial character of the blood stream. No doubt the amoeboid movements and changes in shape of the leucocyte enhance emigration through the blood vessel wall. Thoma asserts unconditionally that his investigations have shown that the amoeboid condition of the protoplasm of the leucocyte is essential to its emigration and its wandering into tissue. It will be observed in other parts of this chapter that many years ago Lawdowsky advocated the same view. It is noted that the leucocyte does not adhere to the surface of the blood vascular endothelia, but to the margin of the plates, i. e., it tends to stick to the interendothelial space. The leucocytes which leave the blood vessels enter the lymph channel. The process of emigration of leucocytes is a normal one. They pass from the blood vessels into the lymph channels through two endothelial membranes as a physiologic process. In the normal blood stream the marginal zone is generally destitute of corpuscles, but frequently leucocytes enter into this corpuscleless region of fluid, and the leucocyte is apt to become adherent to the endothelial wall of the blood vessel, and as the adhesive power of the leucocyte is one of the methods of progress, it is apt to pass out of the vessel. However, distinct proof may be observed of leucocytes in normal tissue and out of normal vessels, by noticing the wandering cell as discovered by Recklinghausen in connective tissue. Hering observed that white corpuscles would wander out of the blood vessels in the frog's web when the blood vessels were, so far as could be noted, normal in their wall and blood current. Hence we may assert here that observation teaches that



the wandering out of the white blood corpuscles or leucocytes from blood vessels in lymph channels (peritoneum) is a normal and physiologic process. This places us on a rock and base for the function of the peritoneum. It is to receive white blood corpuscles, leucocytes from the blood vessels. The emigration of the leucocyte is rapidly increased by disturbance in the blood circulation. The increase of leucocytes in the marginal zone of blood vessels can be noted by producing an irritation

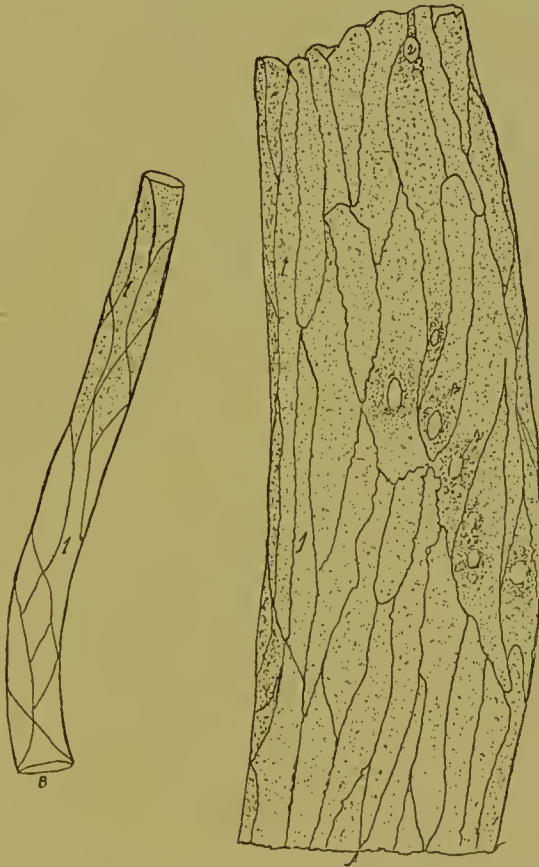


FIG. 124.—(Author.) A, artery of rabbit's mesentery. B, capillary of child's mesentery. A, the artery of the rabbit's mesentery is carefully sketched (oe. 2, ob. 8a, Reichert). 1, endothelium of enormous length. Observe how the endothelia are elongated and of a spindle-shape. 2, 2, nuclei, one cell has two nuclei. The ends of the cells have a peculiar manner of dove-tailing in others. B, a capillary of a child's omentum, two years old. (Ag.  $\text{NO}_3$ ,  $\frac{1}{2}$  per cent. applied.) Only part of the endothelial cells are drawn brown or speckled.

in tissue. Now, when the marginal zone of leucocytes increases they begin to wander through the wall of the blood vessel and hence must induce a greater permeability of the blood vascular interendothelial space. Thus we note that it is a normal process for leucocytes to wander out of a blood vessel, and pathology is but excessive physiology. Again, we note that the blood vascular interendothelial space is normally permeable to leucocytes and then circulatory disturbance increases the permeability of the interendothelial space, so that pathology in both cases is simply excessive or strained physiology. It appears from our experiments that

leucocytes do not wander through the blood vascular interendothelial space entirely aimlessly. The leucocyte does not wander from the vessel promiscuously or universally when larger numbers are called to action, for they seem to tend toward the foreign body as the particles of Berlin blue, especially in the region of the diaphragm. There is one significant affair which may be noted all through this work on the structure and function of the peritoneum, and that is the exceedingly delicate and intimate relations which exist among the three kinds of endothelia, viz.: (a) the peritoneal endothelia; (b) the blood vascular endothelia, and (c) the lymph vascular endothelia. These three kinds of endothelia are inseparable, triumvirate, a "dreibund," each watching for and protecting the other's interest and even existence. If any kind of endothelia are irritated or infected the two others are at once summoned to action. A particle of Berlin blue on the peritoneal endothelia induces immediately the lymph and blood vascular endothelia to make their interendothelial spaces permeable, and a host of leucocytes hasten to bury the Berlin blue out of range of danger. The nerve supply to these kinds of endothelia is in a state of perfect compensatory balances. To irritate one kind of endothelia is like pressing an electric bell button, it rings into action the other two kinds of endothelia. All the leucocytes do not move toward and attack the foreign body in the peritoneum, but the tendency of the leucocytes for the body is so vast that one need not lose sight of the chief campaign. We shall confine ourselves mainly to the peritoneal capillaries which are interposed between the arterial and venous terminals.

The wall of the peritoneal capillaries not acted on by reagents is so transparent that they cannot be seen sufficiently for practical labors. But if stained by ( $\frac{1}{4}$  per cent.) Ag. NO<sub>3</sub>, we at once note that the capillary wall is composed of plates or scales of irregular size and outline. The plate contains a nucleus of variable shape and situation. But the chief point of interest in regard to the blood vascular endothelia is the interendothelial markings, dark irregular lines which indicate the interendothelial space. These dark spaces produced by Ag. NO<sub>3</sub> and light are probably albuminate of silver, doubtless the precipitation of a very fine capillary layer of blood plasma which contains considerable precipitable albumen. None doubts, so far as I am aware, that the dark lines mark the outlines of the endothelial cells. Thoma, who acted as first assistant to J. Arnold of Heidelberg for twelve years, says that he imbibed Arnold's ideas in their labors on the interendothelial substance, "kitt-substance." In 1894 Thoma, in one of the best books on pathology ever published, assuming the same doubt that Arnold did in 1875, announces that he will simply call it "cement substance" without regard to its being more or less firm of fluid. Arnold and Thoma used sul-

phindigodate of soda, first introduced by Heidenhain, or finely ground Chinese ink injected into the blood. Their experiments showed that the finely divided colored matter penetrated between the blood vascular endothelia and passed into the tissue. These experiments showed that the endothelial cells of the blood vessel wall were separated from each



FIG. 125.—(Author.) Artery drawn from frog's mesentery surrounded by lymph spaces. Ag.  $\text{NO}_3$  was applied, and the endothelial surface was brushed or pencilled. 1 and 2 are group endothelia of the vessel wall. Note the arrow-shaped or elongated blood vascular endothelium. The lymph vascular endothelia as 6, 3 and 5 are sinuous and irregular. The upper of the artery shows dark lines across from each side. These transverse dark lines represent the deposit of albuminate of silver between the muscle cells. 4 represents the artery. 5 points to the endothelia covering the lymph vessels or spaces which lie each side of the artery. The ends of the artery are diagrammatic. The vascular endothelia are very variable. Some are ten times as long as they are broad. Notice that the characteristic feature of the endothelia covering lymph vessels or spaces is that of possessing sinuous borders. 6, endothelium covering lymph spaces.

other, and also that the coloring matter did not penetrate the blood vascular endothelial plate. But it does not prove or reveal the nature of the interendothelial substance. They show that the part of the blood vessel wall which allows exit is the interendothelial space. All researches show that the normal vessel walls allow the coloring matter to pass through them from the blood to the tissues, lymph or interstitial spaces.



This process may be called filtration from the blood into the tissue. The coloring matter might pass from the blood to the tissue or lymph channels by another process known as osmosis or diffusion. Osmosis or diffusion would depend on the difference in amount of fluid in the vessels and tissue. So that it must be considered that the processes of filtration and diffusion (osmosis) no doubt share in passage of the colored matter in liquid suspension from the blood vessel to the tissue and lymph spaces. And that filtration and diffusion (osmosis) is chiefly if not entirely carried on by way of the interendothelial space. Arnold uses the term imbibition for the passage of material from the blood vessel to the tissues or lymph spaces.

In this physiologic study of endothelial membranes there must be considered the difference in pressure between the blood and tissue fluid. Also the difference in the composition of the blood must be taken into account. Some, like Pflueger and others, would note that the endothelial membranes have a glandular nature. If so, it must be a peculiar modification of the generally accepted gland tissue. Thoma, in his great work of 1894, assumes that the endothelial membranes of the blood capillaries possess a power of secretion.

One discovery tends to lead to others. When Recklinghausen demonstrated that milk-drops and coloring matter suspended in fluid placed on the serosa of the diaphragm would pass into the subserous lymphatics, it induced much wider investigations by his school of pupils. He showed that the peritoneal serosa possessed certain kinds of rings and spots and stomata and stigmata. Oedmansson was the first to draw cuts of them, noting obligation to Recklinghausen. Then His showed that the endothelia of the lymph vascular system possessed the same stomata and stigmata as the peritoneal serosa does. Finally, the brilliant genius, Conheim, demonstrated that the endothelia of the blood vascular system possess similar stigmata and stomata. Thus it is seen that stomata and stigmata are common to the endothelia of the lymph vascular and blood vascular systems as well as the peritoneal serosa. To the lymph system we relegate the peritoneum. Again, after long microscopic investigations I became convinced from modern reagents, especially suggested by Kolossow, that the endothelia of the peritoneal serosa are bound together by anastomotic protoplasmic processes; it became only a matter of further study to be convinced that the endothelia of the lymph vascular and blood vascular systems were bound together by exactly the same kind of processes of protoplasm. This protoplasmic connection of vascular endothelia does away with the hypothetic cement substance and proves the ground for general study of the vascular and lymph endothelia. We know by many experiments of our own as well as all the recorded ones that white blood corpuscles

(leucocytes) can pass in and out of the peritoneum. We also know that colored granules will pass from the peritoneum (lymph sac) into the subserous lymph sacs. We will note some of the points which show how the leucocytes pass out of the blood vessels, into the lymph spaces. It is recognized that material does not pass out of the vessels through the endothelial plates, but between them. For a proper understanding of the passage of leucocytes through the interendothelial space of the

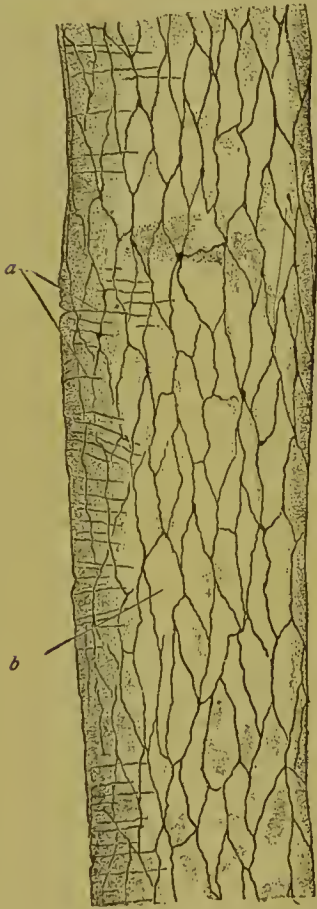


FIG. 126.—(After Stohr, 1894.) Vascular endothelia of a rabbit's mesenteric artery, magnified 260 times. The endothelial membrane is stained with Ag. NO<sub>3</sub>. a, border line of nucleated fibres. b, vascular endothelial cells.



FIG. 127.—(Author.) Capillary from mesentery of rabbit. Ag. NO<sub>3</sub>,  $\frac{1}{2}$  per cent. 1, nucleus. 2, 2, elongated endothelia. (Oc. 4, ob. 3, R.) Note grouping and elongation of endothelia.

blood vascular endothelia many conditions must be taken into account.

1. The leucocyte must be studied as to its action, character and conditions which favor its passage.
2. The condition of the endothelial cell as to the contractibility and expansibility, or in other words its elasticity, must be watched.
3. The interendothelial space with its reorganized structures must be observed with any change that may arise as the leucocyte passes.

Most experimenters have studied this subject by inducing excessive physiology of the parts. The blood vascular endothelia were irritated

so that they would act vigorously which would allow their function to be seen and results observed. In other words, the typical method of study is to induce congestion or inflammation of the parts. When the blood vascular endothelia have implanted on them an inflammatory process one can kill the animals at different stages of the process and then observe the condition of the endothelial space and the leucocyte itself. I pursued this method entirely in experiments. It is very plain that in inflammations the interendothelial space is more affected than the endothelia plate, for often in inflammatory processes the Ag.  $\text{NO}_3$  stains wider lines, more stomata and stigmata appear which are, however, quite irregular. Hence, experiment teaches that the physiologic seat of the blood vascular endothelia is the interendothelial space. Conheim attracted much attention in 1867 when he announced that the red and white blood corpuscles would pass out of blood vessels at certain points, stomata, and Arnold in the same year demonstrated diapedesis. When colored granules are injected into the blood vessels the outlines of the blood vascular endothelia are not only marked by the outlines of the adjacent lymph vascular system, but the granules are deposited. It has been well recognized from the experiments of Recklinghausen, Reitz, Ponfick, Auerbach, Hoffmann, Langerhans, Conheim, Eberth, Chrzonszczewsky and his pupils, Ludwig and his pupils and especially J. Arnold that colored granules injected into the blood will pass into the lymphatic channels. And all experiments show that the material passes by way of the interendothelial space. Hence, the chief study of the blood vessel belonging to the peritoneum will be directed to the vascular endothelial tube. Aeby, Pouyes, Alferow and Loret have also demonstrated that the white corpuscles pass out of the vessels by way of the interendothelial space. Arnold reports some of the most valuable experiments and shows by means of very elegant cuts that he has many times observed the white blood corpuscles leucocytes clamped between the endothelial cells. Experiments also show that colored material injected into the connective tissue will be deposited.

The interendothelial space of the blood vascular and lymph vascular and peritoneal endothelia shows that the mode of travel of fluids through endothelial membranes in the body is by way of the various interendothelial spaces. Experiment has fully demonstrated that the substances finally divided and suspended in fluids pass directly through the interendothelial spaces or to the peritoneal cavity. The path being determined, it then becomes a matter of consideration as to the methods of travel in the path. When Recklinghausen, Aeby, Eberth, Auerbach, His and others showed that endothelial membranes were constructed of endothelial cells, it produced various opinions as to the nature of the endothelial space. Some claimed it was a fluid or semi-fluid cement



substance. Others claimed that the cells had a more intimate connection than any cement substance could afford. Still others held that the looseness of the connection of the endothelial cells was demonstrated by finely divided colored granules rapidly passing between them. Still other investigators said that the  $\text{Ag. NO}_3$  simply precipitated the albuminous fluid that lay in the interendothelial depressions or troughs. It cannot be said that the wanderings out of the white corpuscles is due to excessive pressure of the injected fluid, for it is performed in a physiologic method. From the close study of the interendothelial space in the

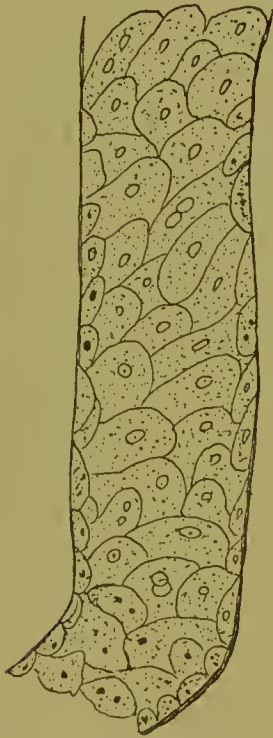


FIG. 128.—(Author) Drawn from a perisalpinx, human (oc. ob. 8a, R.). It is a young blood vessel forming among the extensive exudates. The surface is covered with thousands of white blood corpuscles. The patient had enormous double pyosalpinx and the pus bathed this surface.

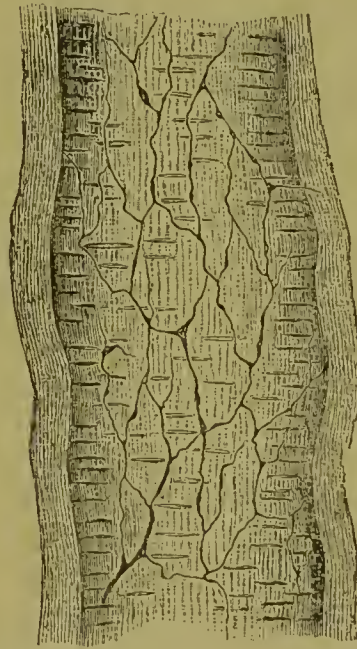


FIG. 129.—(Handbook of Phys. Lab., Vol. II., 1873.) Horizontal preparation of mesentery of frog, treated with gold chloride, showing the plexus, giving a surface view of fibres in the adventitia of a large artery. (Oc. 3, obj. 7.)

blood vessels arose the terms *stomata* and *stigmata* as directly referred to the blood vessels. (The complicated structures known as *stomata vera* on the peritoneum, which were discovered and written about by Recklinghausen, Oedmansson, Schweigger-Seidel, Dogiel and Klein, have a different meaning.) The terms *stomata* and *stigmata* are generally referred to Arnold as their originator. He seems to indicate by these terms larger and smaller points on the interendothelial lines of the blood vascular system. From the time of the discovery of these *stomata* and *stigmata* there exist two opinions as to their signification. One view is that they owe their origin to their method of preparation, and

hence are artificial products. The other view is that they are normal structures belonging to the interendothelial lines of blood vessels. The argument against the stomata or stigmata being anatomic structures with physiologic function, is their irregular appearance and irregular distribution. They may be very numerous or they may almost entirely fail. The irregularity of distribution argues in favor of the fact that they are local expansions of the interendothelial space, due to increased blood pressure or irregular endothelial plate contraction. The changeable localities of the structure argue that they may not be preformed openings or stable structures of the blood vascular endothelia. The function of the blood vascular endothelial structures known as stomata and stigmata must be looked on as a relation between blood vessels contents and adjacent tissue and between blood vessels contents and lymph vessel contents. The view that the above is their function rests on the fact that colored granules, finely divided and suspended in a fluid, which being injected in the blood vessel will be found deposited in the tissue adjacent to the blood vessels and in the lymph vessels adjacent to the blood vessel. In other words, the stigmata of the blood vascular endothelia shows an intimate connection to the subject of nourishment. In experimentation, the question must always be kept in view whether the results gained by experiment are really physiologic or pathologic or whether we are dealing with a mongrel, partly physiologic and partly pathologic. For, our experiments demonstrated to us that whatever induced the leucocytes to pass out of the blood vessels seemed to increase their number in the blood (or perhaps in the lymph spaces). The leucocytes seemed to stand guard over the organization in such a way that if one leucocyte was called out thousands seemed to follow, perchance, as a reserve force. Of course, we cannot and must not be too critical, for an experiment itself is almost always excessively physiologic. To inject into the blood vessel colored granules cannot be far from irritation. Something beyond pure physiology is added, but in general it does not destroy practical results. It is only when the experiments become carried beyond the physiologic function that it fails to show physiology. There is no doubt but that the experiments on the blood vascular endothelial membranes have been carried out sufficiently delicate to demonstrate beyond a doubt that the physiology of the endothelial membranes belongs to the interendothelial space almost exclusively. Doubtless osmosis (and some diffusion) will occur through the endothelial plate of the blood vascular system, especially with high blood pressure or when the blood tension in the vessel is higher than in the tissue, or the fluid in the vessels thinner than in the tissue.

The interendothelial substance (space) has concerned investigators for many years. Twenty years ago Julius Arnold, professor of path-

ology at Heidelberg, who carefully studied what he called the "kitt-substance" (interendothelial substance), wisely and prophetically remarked that the connecting of the endothelial cells was not the chief office of the interendothelial substance, but that between the endothelia run processes which play a prominent role. It was noted then (1876)



FIG. 130.—(Author.) Woman's omentum majus (oc. 4, ob. 3.). 1, 2, 3, lymph capillary or sinus; 9, 10, 11, vacuolated cells or lymph sinuses in formation; 12, 13, 14, germinal endothelia around the vacuolated cells intensely brown (a) artery; (b) and (c) branches; 5, 6, 7, 8, lymph capillaries, with young common surface endothelia. This cut shows the panoramic scenes of the omentum in woman of vacuolation, germination and stomata vera. Stomata vera and stomata spuria present. Here may be seen common peritoneal endothelia covering lymph vessels and germinating endothelia all within one microscopic field.

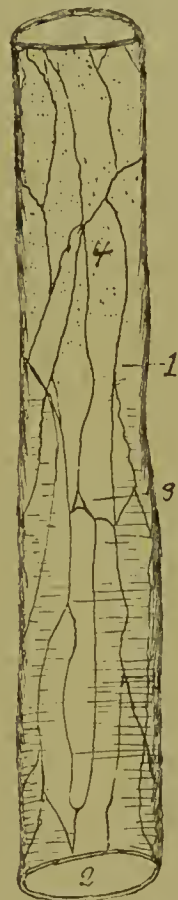


FIG. 131.—(Author.) Drawn from mesentery of frog (Oc 4, ob. 3, Reichert). This artery was carefully sketched and shows very irregular and elongated endothelia. 1, a long, spindle-shaped vascular endothelium; 2, vessel itself; 3, stoma verum (or spuria). 4, shows the endothelium browned by the silver (speckled), at the upper end of the vessels. The lower end of the vessels shows the dark transverse lines which are the albuminate deposits of silver between the circular muscle cells. Irregularity and elongation of endothelia is prominent in this vessel.

that circulatory and assimilatory disturbances chiefly belong to the interendothelial substance (space). In 1867 Conheim first noted that in certain points (stomata) in the vessels, red and white corpuscles would pass out of the vessels and be naturally attributed to the stomata in the interendothelial substance (space), as also did Samuel. So that Arnold's



conception of the interendothelial substance twenty years ago was not widely remote from the modern view, which is, that to alteration of the interendothelial substance (space) must we look for function in the endothelial membrane, the passing of blood corpuscles or fluids through its tissue. All who experiment may observe that the interendothelial change or function is manifest, and this idea remains the same whether we labor with blood vascular, lymph vascular or peritoneal endothelia. Arnold attempted to settle the function of the interendothelial space by injection of sodium sulphate colored with indigo into the blood directly. He secured beautiful pictures showing the deposit of the coloring matter in the interendothelial substance (space). Ag.  $\text{NO}_3$ , directly applied, shows that it attacks chiefly the interendothelial space, so that both of these experiments direct our attention to the interendothelial space as

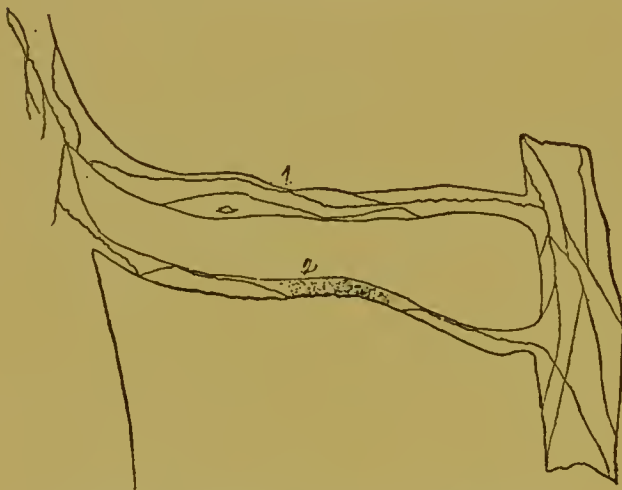


FIG. 132.—(Author.) From young dog's omentum to show growing capillaries 1 and 2. At 2, solid protoplasm not quite pervious for the blood stream. 1, is completely pervious to the blood stream.



FIG. 133.—(Handbook of Phys. Lab., 1873.) A capillary blood vessel, the cavity of which is extending into a branched connective tissue cell.

the structure through which the interendothelial membrane exercises its function, and that the blood and lymph vascular and peritoneal endothelial membranes act similarly. Arnold seized on the experimental method by injecting coloring matter in the blood, and then to observe where the colored granules became deposited. It was by this method that Arnold became convinced that the physiologic seat of the vascular or peritoneal endothelia is in the interendothelial substance (space). Arnold at that time asked the question, whether there really was any fluid substance between the endothelia. In his experiments of injecting coloring matter into the blood, he found that the colored granules soon marked out the outline of the blood vascular endothelia, and also that the coloring matter rapidly passed out of the blood vessel into the lymph channels and there distinctly marked the outlines of lymph vascular endothelia.

This led Arnold to state that the function of the endothelial membranes was shown by experiment to lie in the interendothelial (space). Some older authors thought that the endothelial lines were simply an imbibition phenomenon, because the cover-plate of the endothelial cell and its nucleus share comparatively little in the function of distributing coloring matter. It is proved by many and varied experiments that the endothelial cell-plate and its nucleus plays comparatively no role in the function of endothelial membranes. Experiment distinctly shows that colored granules passed out of a blood vessel between the interendothelial cells, i.e., through the interendothelial space. It has been discussed for over twenty-five years that the changes produced by



FIG. 134.—(Author.) Capillary from human omentum of child 2 years, showing surface endothelia. Ag.  $\text{NO}_3$   $\frac{1}{2}$  per cent. applied. Its whole wall consists of endothelial plates joined edge to edge. 1, not browned endothelia; 2, browned endothelia.



FIG. 135.—(Handbook for Phys. Lab., Vol. II., 1873.) Horizontal preparation of mesentery of frog treated with ehloride of gold, giving the surface-view of a large vein with the plexus of nucleated non-medullated nerve-fibres which lie in the adventitia of the vessel.

passage of corpuscles through the interendothelial substance (space) may be purely a local widening of this hypothetical substance at the exact point where the spot or ring is located. Arnold, whose excellent labor in regard to the interendothelial substance is a lasting example of industry, concluded that the endothelia were loosely bound together, that the small space between the endothelia is filled with fluid or

at least a semi-fluid substance which allows the passage of finely divided colored granules suspended in fluid, that the interendothelial substance changes according to the expansion or contraction of the endothelial membrane, and that the position of the endothelial plates in relation to each other is a changeable one.

The development of the blood vessels in the peritoneum is of much interest, but we will only make a few remarks in regard to it. The capillaries develop out of branches connective cells. The fluid in the tissue has an oscillating movement, and it appears to hollow out the connective tissue cells and produce the blood channels. There are vacuoles formed in the midst of the connective tissue cell, but it requires a long time for the connective tissue cell to become like an endothelial plate forming the vascular endothelial tube. It is very unlike the vacuolation to form lymph channels, for in lymph vacuolation the cell quickly

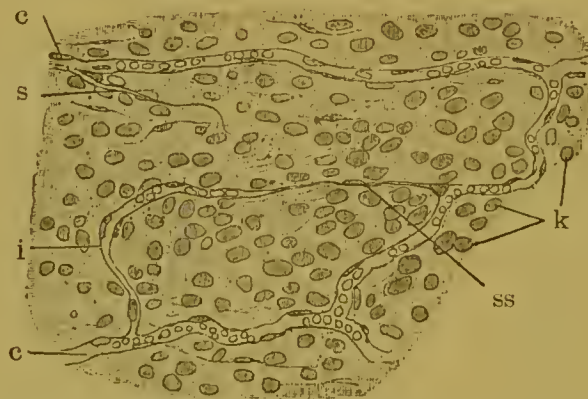


FIG. 136.—(Stohr, 1894.) Flat surface of a piece of omentum majus of a seven-day-old rabbit magnified 240 times. c, blood capillary, parts of which contain blood corpuscles. s, sprout of a capillary with a solid outrunner. i, young capillary, for the most part hollow. ss, still solid. k, nucleus of peritoneal endothelia.

assumes an endothelial character. Thoma speaks of the blood vascular endothelia as a secreting surface, that is, a surface which must become involved in nutrition. Besides, the endothelial walls of the capillaries would then secrete their own fluid. The blood spaces are formed in intercellular spaces which are filled with a clear fluid. As the fluid has movement, it is a kind of circulation. With this clear moving fluid in the intercellular spaces the red blood corpuscles soon become mixed. According to Thoma, the rows of cells from which the capillary tubes develop have a secretory power. The cells forming the walls of the capillary tube secrete a clear fluid. This fluid distends the tube, making the fluid pressure in the tube higher than in the adjacent tissue. It is found that the surface of the connective tissue cell which is turned toward the lumen of blood capillary assumes a refractile appearance like other secretory surfaces. As the capillary tubes advance in the connective tissue cell it seems they become vacuolation and the surface



turned toward the capillary lumen secretes fluid under a fairly high tension. The capillary blood vessel thus slowly advances in the tissue, and in quite young animals one can sometimes observe where two terminals of blood capillaries are about to meet with a very short impervious cord of cells between the two. In one cut (No. 132) which I drew from a very young dog's omentum, this condition may be clearly seen. With the idea that the blood vascular endothelia form a secretory surface which transforms nutrient material into other products, it presents reasonable views as to the development of capillaries. The most beautiful labors on the development of the blood capillaries is that by J. Arnold, of Heidelberg, and his celebrated assistant, Richard Thoma, who labored 12 years with Professor Arnold and then was professor of pathology at Dorpet for 10 years, when he resigned. The above two writers and investigators show by elegant cuts how the blood capillaries advance into



FIG. 137.—(Author.) Drawn from the ligamentum suspensorium hepatis (human) treated with Ag. NO<sub>3</sub>. 1, 2, 3, 4, shows very granular cells—stomata vera. Observe the grouping around these granular cells. 5, stoma spurium. It appears that some portions of the granular cells are simply seen shimmering through the transparent endothelial plate. 5 may be a constricted off loop of a cell.

connective tissue cell by hollowing them out. The capillaries are protoplasmic tubes composed only of a single layer of flattened connective tissue cells transformed in blood vascular endothelial plates. No muscles exist in the capillaries. After staining with Ag. NO<sub>3</sub> the spindle-shaped, nucleated, endothelial plate can be plainly observed with its irregular outlined interendothelial space. The capillaries of the omentum of a very young animal are the best kind of material for general observations. Careful microscopic examination will be rewarded by noting the gradual coalescence of blood spaces into blood capillaries. To show that the blood capillaries of the peritoneum are formed of connective tissue cells, we can plainly see that the connective tissue cell is distinctly connected with the capillary wall. This will be illustrated in several cuts. Thoma has formulated some general laws of the growth

of blood vessels which we will here insert in order to conclude the subject. They are:

1. The increase in size of the lumen of the vessel, or what is the same thing, the increase in the surface of the vessel wall, depends upon the rate of the blood current.

2. The growth of the thickness of the vessel wall is dependent upon its tension. Further, the tension of the wall is dependent on the diameter of the lumen of the vessel and upon the blood pressure.

3. Increase of blood pressure in the capillary areas leads to new formation of capillaries.

The above three general propositions rest on histo-mechanical principles and are very important in the study of physiologic and pathologic conditions of blood vessels.

These general laws explain obliteration and increase of blood vessels, and especially the new formation of capillaries under high blood pressure as exudates and inflammatory processes in the peritoneum. These principles show that it is the organ itself which determines the rate, quantity and pressure of blood in its incoming and outgoing vessels. The blood vascular endothelium consists of a cover-plate which does not seem so indurated as the cover-plate of the peritoneum. Also if underlying protoplasm with its nucleus, this is the essential portion of the endothelial cell.

#### CONCLUSIONS.

1. The smallest blood vascular tube is composed of simply nucleated, elastic endothelial plates connected together at their edges by anastomotic, protoplasmic processes. The protoplasmic processes cross the interendothelial space at varying intervals.

2. The interendothelial line is a network space and not a fluid nor semi-fluid substance.

3. The endothelial plates of the blood vascular system are but slightly engaged in the exit of corpuscles or fluid from the vessel.

4. The interendothelial space is crossed by anastomotic protoplasmic processes which bind the endothelial plates in a colony. The processes leave intervals of space between them, rings and dots.

5. The seat of physiology of the blood vessel wall is chiefly in the interendothelial space.

6. Fluids and solids (corpuscles) pass from the lumen of the blood vessel into the adjacent tissue or lymph channel by way of the interendothelial space.

7. It is probable that the endothelial plate of the blood vascular system has a secretory surface which is concerned in nourishment which transmits to the blood products and to the tissue products.

8. The majority of authors agree that stigmata and stomata arise as

conditions to allow the leucocyte to pass through the interendothelial space. The minority insist on the stomata as preformed openings, as organized channels.

9. My experiments seem to show that whatever induces the leucocyte to pass out of the blood vessel tends to rapidly increase the leucocyte in number.

10. The apertures, stomata or stigmata observed in the interendothelial space are considered to be the products of exit of the leucocyte by some authors.

11. The blood vascular endothelia consists of (a) a cover-plate (Kolossow), a smooth indurated portion of protoplasm; (b) of a subjacent protoplasm containing a nucleus, and also the projecting protoplasm which unites the cells into colonies.

12. The endothelial tube is the constant portion of all blood vessels. The migration of leucocytes from the blood vessels to the adjacent tissue or lymph channels is a physiologic process.



## CHAPTER VI.

### THE LYMPHATICS OF THE PERITONEUM.

The knowledge that a man can use is the only real knowledge: the only knowledge that has life and growth in it and converts itself into practical power. The rest hangs like dust about the brain, or dries like raindrops off the stones.—*Froude.*

The peritoneum itself is a lymph sac, and hence the significant feature in its walls is the abundance of lymph structures. The interstitial space known as the peritoneal cavity originated by interstitial fluid pressure and independent motion of the body wall and viscera. The coalescence and disappearance of its partitions with consequent enlargement of its size was an evolutionary process. With the growing of mobile viscera and independent action of the body-wall, the peritoneum not only assumed greater dimensions, but a capacity to facilitate visceral movement. The lymphatic system is a secondary vascular system, and only belongs to the vertebrates. It is super-added for the purpose of nourishing tissue in a fluid medium, i.e., to float to cells requisite food and to float away effete material. Lymphatics are numerous only when blood vessels are numerous. In this article I shall not consider the lymphatics of the intestines, the lacteals as belonging to the peritoneum. They simply run between its mesenterial blades, but are not of it. Having made extensive microscopic study of the lymphatics of the peritoneum of man as well as of the rabbit, dog and several other animals during the past two years, I wish to present here some views in regard to the lymph spaces or interstitial spaces.

The lymphatic system of the peritoneum consists of:

1. Interstitial (lymph) spaces.
2. Non-valved capillaries.
3. Valved lymphatic channels.

We shall first consider the interstitial spaces of the peritoneum. These spaces have been called lymph spaces, lymph sinuses or perivascular spaces. The reason that investigators have placed so little importance on the subperitoneal interstitial spaces is because they have studied it chiefly from the human. To be impressed with interstitial spaces of the peritoneum, one has only to study the amphibia, especially the turtle. In the turtle with Ag. NO<sub>3</sub> and a delicate brush to gently remove the peritoneal endothelia, we can see with ease the vast interstitial

spaces and the wide perivascular spaces in the subendothelial tissue. The endothelial or lymph sacs gradually lessen in size or prominence, but not in number, as we progress along the ascending scale of vertebral life. We shall place especial stress on the interstitial spaces in the subperitoneal tissue on account of its vastness, on account of the significant role it must play in the absorption of peritoneal fluid,



FIG. 138.—(Author.) Drawn from the cisterna lymphatica magna of frog (oc. 2, obj. 8a, Reichert). This figure shows well the grouping of endothelia around a stoma verum. 1 and 2 point to nuclei of the germinal, granular cell. 3, 8, lining the stoma verum. 4, 4, 4, stomata spuria. 5, points to a black mass; no doubt covering the granular germinating cells which line the stoma verum. It may be observed that at 8 the granular cell appears below the endothelium and shimmers through it.

on account of the importance of its function in nourishment and on account of its difference in anatomical limitation. We think the interstitial spaces of the peritoneum sufficiently and significantly different to be given a separate consideration. We have to a certain extent returned to the old views of the physiologists who found from experiments that the whole connective tissue is a network of channels. They announced that its interstices were directly or indirectly connected with the lymph capillaries and valved lymph trunks. These old ex-

perimenters considered the lymphatic system pre-eminently a circulatory system of the connective tissue. This lymphatic system is found in all connective tissue. Revived experiments are again leading us to similar conclusions. The old physiologists claimed that the lymphatic system in the serous membranes (peritoneum) was a great absorbent system. I believe from my experiments and microscopical labors on the subperitoneal tissue, extending over several years, that the old physiologist is correct. The complicated connection of the lymphatic system of the peritoneum with the blood vascular system is so intimate that one cannot be lost sight of in considering the other.

The discussion of the various modes of origin of the lymphatics has been extensive. We may say briefly that three general modes of origin are acknowledged:

1. The lymphatics arise by stomata which maintain an open and direct communication between the peritoneum (interstitial space) and the lymphatic vessel. The stomata are organized channels or apertures situated at the common junction of several endothelial plates and are lined by granular, polyhedral, nucleated cells, which stain deeply on the application of  $\text{Ag. NO}_3$ . The stomata allow the lymph fluids to pass and re-pass from the peritoneal to the lymph vessels. The peritoneum may be considered as a large lymph lacuna. Owing to the stomata maintaining a direct, open communication between the large lymph lacunae (the peritoneum), lymph is not allowed to accumulate, as muscular action and bodily motion as well as intra-abdominal pressure induce the lymph to flow through the stomata into the lymph vessels.

2. The lymphatics arise by lacunae or interstitial spaces. The origin of the lymphatics by the methods of interstitial spaces is typically represented in the subperitoneal tissue. This mode is best observed in the amphibia, especially in the frog and turtle. In thin, flat membranes, as the peritoneum or diaphragm (centrum tendineum), the lymphatic vessels form a network situated generally in one plane, but they may be located in several planes and united by vertical vessels or channels, as in the centrum tendineum of mammals. The interstitial spaces or lymphatics of origin are very various in shape, size and distribution, and depend more on the quantity of fluid contained than on anatomic limitations. Lymph is collected in the interstitial spaces from the subperitoneal tissue and conveyed to valved lymph channels. The lacuna mode of origin of lymphatics was described by Ludwig and Tomsa. The interstitial spaces penetrate the interstices of the extensive subperitoneal tissue. They are clefts, gaps or slits located between cells and elementary tissue and lined by endothelial cells.

3. Another mode of origin of lymphatics is termed the plexiform.



This differs so little from that termed interstitial that we will not consume space in describing it.

Gaskell claims that some of the smaller lymph vessels have elastic fibres attached to their wall in such manner that the elastic fibres restore the lymph lumen after pressure has collapsed it. The relation of the lymphatics of origin to the cells and cell-spaces in the subperitoneal tissue is best studied by silver staining and gold staining and producing oedema in the tissue by peritoneal injections. The cells of the subperitoneal tissue lie in spaces in the ground substance. The cells



FIG. 139.—(After Klein.) Represents a network of lymphatic channels on the peritoneal surface of the diaphragm of a rabbit, prepared with silver nitrate. (a) is a large lymph vessel; (b) shows lymph capillaries, and (c) the beginnings of the capillaries.

partially or almost wholly fill the spaces. The cells and cell-spaces form a fine inter-communicating network. The spaces, containing one or more cells, are vacuoles in the ground substance of the subperitoneal tissue. The spaces in the ground substance present a close relation to the lymph vessels, as both vessels and spaces are lined by flattened endothelial cells joined by an interendothelial space. The wall of the space, i.e., the single layer of endothelial cells, is so thin that it doubtless offers little resistance to the passage of fluids or corpuscles.

It appears to the author that cell-spaces of the subperitoneal tissue, especially that in the diaphragm, where it is typically to be observed, are entirely empty except fluid. It may be that this is a delusion caused by the cell in the cell-space becoming closely applied to the wall of the ground substance. Staining reagents (Ag. and Au.) make the subperitoneal tissue present wide spaces and spaces closed or at least only represented by a line or slit. In a state of oedema the spaces of

the subperitoneal tissue become more or less distended, when the spaces distended by fluid may appear larger than the intervening cells.

In the subperitoneal tissue when the juice canals, lymphatic canaliculi, which correspond to the cell-spaces, are almost completely filled by protoplasmic cells, the lymph fluid may find its way by circulating between the clefts of the cells and the ground substance which surround them. With less completely filled cell-spaces, of course more free lymph and corpuscular circulation occurs. It appears from the author's experiments that the whole of the subperitoneal tissue, but especially the dorsal, is permeable by fluids.

The development of lymphatics is the same as the development of blood vessels. Thoma and Klein have done excellent labors in this field. In the rabbit one can observe the formation of the lymph vessels and spaces quite progressively. The vacuole is formed in a cell of connective tissue and gradually enlarges until it becomes a considerable cavity filled with fluid. The wall of the cavity is composed of the protoplasm of the cell thinned out so as to produce a cyst or vesicle. Doubtless it is this condition which induced Professor Thoma to assert that the vascular surface of the endothelia is a secretory surface. After the connective tissue cell has expanded into a vesicle holding lymph, the internal portion of the protoplasm of the cell bulges inward and breaks off and becomes a lymph corpuscle, or more likely, lymph corpuscles wander in through the wall of the expanded vesicle. Very beautiful pictures may be observed in the omentum of the rabbit, the rat, and other mammals in regard to the developing lymphatics in the omentum. The vacuolation may be noted in all stages of expansion in the various portions of the peritoneum, but especially in the omentum. The nucleus of the cells multiply and the protoplasmic portion of the cell expands into an endothelial cell-plate which responds to silver reaction. In the case of lymphatic spaces or vessels the border of the endothelial cell becomes sinuous in outline. The lymphatic vessels and spaces are formed by the vesicles becoming connected to each other by processes into which their cavities extend in the contained fluid. In the breeding season (and even at other times) I have noted in the frog and turtle endothelial cells prominently marked by cilia. The best portion of the peritoneum to secure the ciliated cells is in the mesentery of the ova sac.

To illustrate the unsettled views in regard to the relations of the blood vessels and interstitial spaces, Prof. Heidenhain, a leader in thought and investigation, says in one article it is inconceivable that under normal circumstances a part of the lymph should be returned directly to the blood, while in another article he notes that it is his conviction that the blood vessels are the essential paths of absorption of



fluid from the peritoneal cavity. Hence, we may sum up by saying that the subperitoneal tissue is permeated by a system of interstitial spaces which contain the fluid that bathes the tissue. The interstitial spaces in structure and function are such distinct and prominent features that they should be considered separate if not independent. At least they should have full consideration as a characteristic apparatus of the subperitoneal tissue. The interstitial spaces receive fluid from the blood vessels and are depleted chiefly by the lymph vascular system,

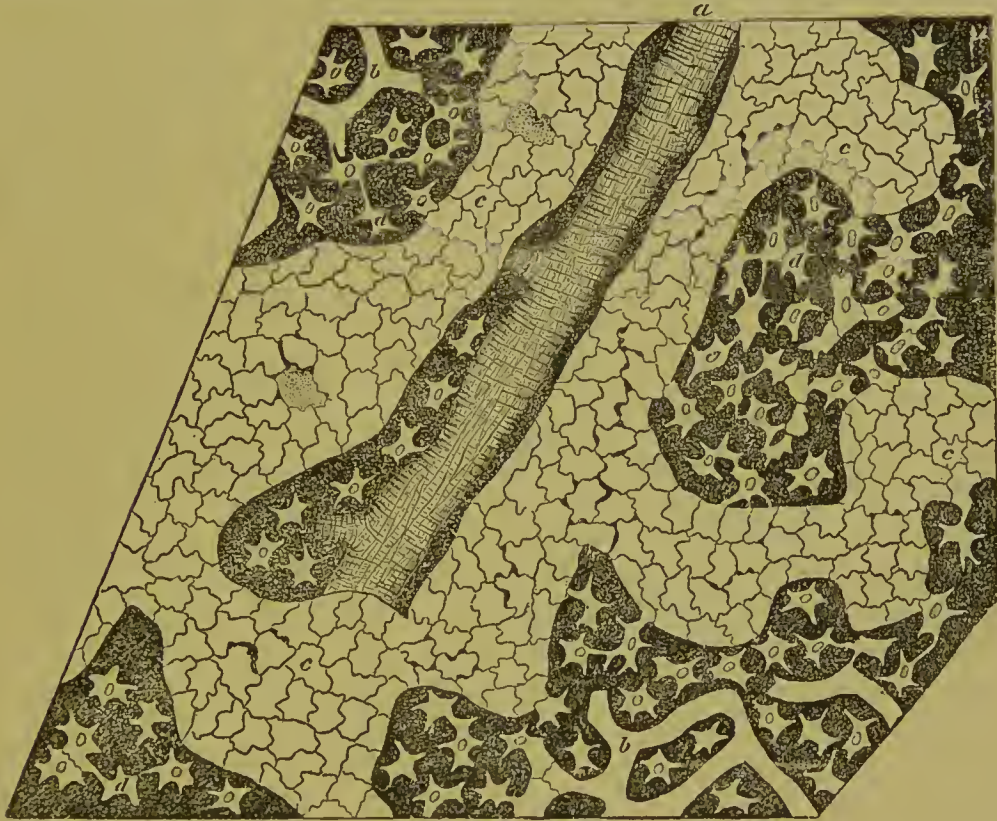


FIG. 140.—(After Handbook for Phys. Lab., Vol. II., 1873.) Omentum of rabbit, pencilled and colored in silver. (a) Artery. (b) Capillary blood vessel. (c) Network of lymphatics, reorganized as lymph capillaries by their sinuous endothelium and the absence of valves. (d) Lymphatic canaliculi of the ground substance; in most of them the nuclei of the cells contained in them are seen. (Oc. 3, obj. 5. Tube half drawn out.) Observe here the peri-vascular lymph spaces of the artery (a.)

but also by the secretory glands. Doubtless, some of the tissue fluid in the interstitial spaces of the subperitoneal tissue is returned directly to the blood vessels. We have noted elsewhere that the fluid in the interstitial spaces of the subperitoneal tissue is controlled by the factors (a) stomata, (b) imbibition, (c) osmosis, (d) filtration (mechanical pressure) and (e) vital processes. It may be assumed that the blood pressure is higher than the tissue fluid in the interstitial spaces and that the pressure in the interstitial spaces is higher than it is in the lymph channels. This assumption would explain, to a certain extent, the constant movement of the tissue fluid and lymph.



Later labors have made general the view that the peritoneum is an important part of the lymphatic system. It is, in fact, a membranous expansion of a portion of the lymphatic system. Before me lies Virchow's cellular pathology, 2nd edition, 1858, in which he originated the view that the starting-point of the lymphatics is from hollow anastomosing cells, and then noted that such a view must be regarded as a new acquisition to our knowledge. However, Von Recklinghausen showed, by the aid of Ag. NO<sub>3</sub>, that the interstitial spaces were lined by endothelial cells. He observed the passage of milk and fine granules through openings in the central tendon of the diaphragm from the peritoneal to the pleural surface. He also announced the discovery of a system of canaliculi in the connective tissue which he termed "saftkanalchen," or juice canals. Later he announced the theory that the connective tissue is traversed by serous canaliculi or plasmatic channels which are directly continuous with the lymphatic vessels.

In the subperitoneal tissue innumerable serous canals lie buried. The lymphatics and lymph or interstitial spaces have been treated generally as if they were one and the same system. The lymph spaces have been considered the roots of the lymphatics, but we must insist on the interstitial spaces being endowed with a vastly wider office than merely the roots of the lymph channels. It is true the interstitial spaces of the subperitoneal tissue are fed by the blood capillaries and drained by the lymph channels, but in the interstitial spaces assimilation, growth, reproduction and decay of cells occur. The interstitial (lymph) spaces in the subperitoneal tissue are of various shapes and sizes. They are gaps or clefts, of the same nature as the peritoneum itself, situated between tissue elements. The peritoneal tissue is very loose in the dorsal region, and hence the interstitial spaces are relatively larger. When the tissue is dense the interstitial spaces are relatively small.

The innumerable shapes and sizes of interstitial spaces may be imagined by a consideration of the various kinds of tissue, as muscle, tendon and fat cells. Some interstitial spaces are so large that an endothelial lining can be demonstrated and the spaces communicate directly with each other. The loose, spongy, subperitoneal tissue represents the most typical interstitial spaces, as can be proven by examining it in the naturally contracted state and again in a state of oedema due to absorption of peritoneal fluids. This I did often, and was astonished to find how wide apart the elements could be forced by the fluid. The forcing apart of the cellular elements in the subperitoneal tissue by the absorption of fluids from the peritoneal cavity only demonstrated the function and capacity of the interstitial spaces. I have noted this fact many times in oedematous tissue. In these

same specimens the direct connection of the lymphatic channels with the serous canals of the connective tissue was also evident.

The variable quality of fluid in the interstitial tissue contains the nutrient as well as the waste products of the adjacent cells. After large injections into the peritoneal cavity with plenty of time for the subperitoneal tissue to absorb, it is manifest what enormous quantities of fluid the loose, subperitoneal tissue will accommodate.

A curious feature about the subperitoneal interstitial spaces is that they do not seem to possess a distinct anatomical limitation, but have the capacity to accommodate large quantities of fluid by appropriating the wall of other spaces to a considerable extent. The large subperitoneal interstitial spaces can only be realized by actual experiment to test the quantity of fluid they will really accommodate.

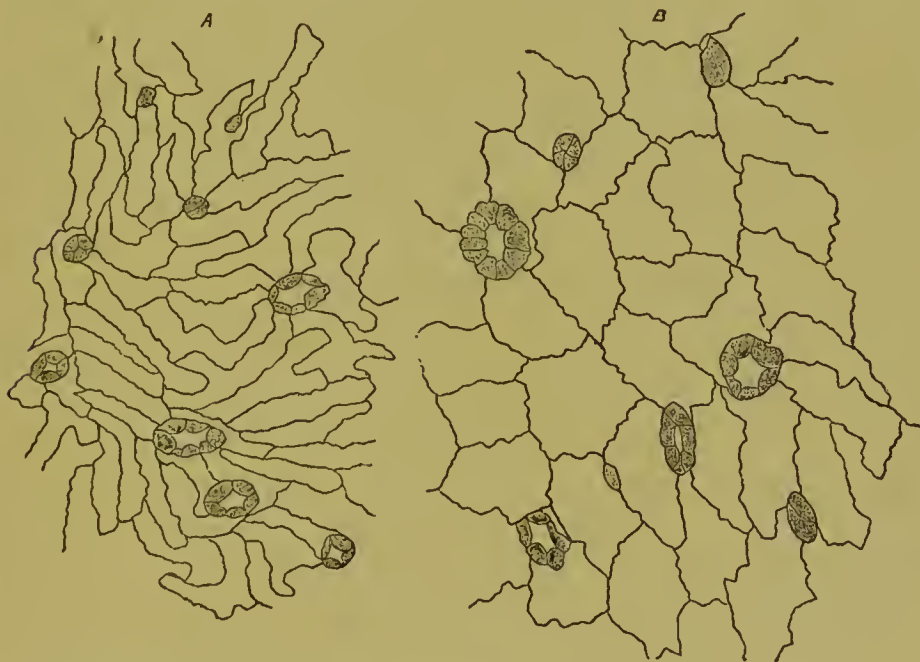


FIG. 141.—(Handbook for Phys. Lab., Vol. II., 1873.) Septum cisternae lymphaticae magnae of frog, colored with silver. A, View of peritoneal surface. B, View of surface of lymph sac. The stomata, some of which are open, some collapsed, are surrounded by germinating endothelium, which is ciliated if the subject is a female. (Oc. 3, obj. 5.)

It is due to the direct connection of the subperitoneal interstitial spaces and their wonderful elasticity that large quantities of fluid can be repeatedly received and passed on to adjacent spaces by muscular action and other forms of motive propelling forces. In the interstitial spaces, especially in the amphibia, as is noticed in the cut presented, on the turtle the blood capillaries are imbedded. It is plain that the larger blood and lymph vessels are separated from each other by two layers of endothelial cells, but where a blood vascular capillary passes through the wider interstitial space it is only separated from the lymph

by a simple layer of endothelial cells. This fact will show the intimate relations of the blood and lymph vascular systems. For, when an exceedingly thin-walled tube passes through a large fluid space, interchange of fluid is quite evident. In the subperitoneal interstitial lymph sacs, the blood capillaries do not enter directly into the lymph capillaries. The fluid leaves the blood capillary first to pass into the interstitial space, and from the interstitial space the fluid enters the lymph capillary. There is as a rule one or more interstitial spaces existing between the blood capillary and the lymph capillary. This is most typically noted in turtle's subperitoneal tissue, also it may be noted in the rabbit. The reader may well remember that one of the characteristics of the interstitial spaces of the subperitoneal tissue is irregularity of shape, variation in size and elastic accommodations of the various quantities of fluid. The idea that the lymphatic channels or vessels constitute one part of the lymphatic system and the lymph spaces, or better the interstitial spaces, constitute the other is an old one, perhaps first suggested in precise terms by that keen genius, Leydig, who claimed that a system of tubules were intercalated between the blood capillaries on the one hand and the lymphatic capillaries on the other.

Virchow and Donders advocated that the stellate connective tissue corpuscles formed by fusion of their membranes a continuous system of tubules which might be termed a plasmatic vascular system. Virchow, Donders and Koelliker considered the plasmatic vascular tubes, or the lymphatic system, as closed. But Ludwig and Bruecke maintained that the roots of the lymphatics of the interstitial spaces were destitute of a membrane and simply arose from the interstitial spaces or from the so-called lacunae. Parts of each of the above views are still maintained. A very significant matter was demonstrated by Mascagni and Folmann, who injected the lymphatics with mercury and found such a complete penetration of connective tissue by the mercury that they arrived at the conclusion that the connective tissue consists of a close plexus of lymphatics and that the solid tissue constitutes only small trabeculae and septa between them. However, it has long been known that there existed what we today consider best to term "interstitial spaces." This system of interstitial spaces is fed on the one hand by blood capillaries and depleted on the other by lymph capillaries. The blood vessels and the lymph vessels are the fluid-carrying vessels to and from this system of interstitial spaces. Both of the fluid-carrying vessels have several common features, viz.: (a) each tube has a distinct, constant direction for its current; (b) it has a limited capacity; (c) each one has a lining of endothelial membrane with, perhaps, muscular and fibrous addition; (d) with nerves to control the calibre; (e) each tube is endowed with



stomata (or the capacity to form them for the purpose of receiving or giving up fluids).

These two tubular systems differ in the fact that the blood vascular capillary carries fresh nourishing food to the interstitial space, while the lymph vascular capillary carries away debris and effete material. The great difference between the two fluid-transporting tubular systems and the system of interstitial spaces lies in their function. The function of the two tubular systems (lymph and blood) is chiefly transportation of fluids.

The chief function of the interstitial spaces is nutrition. The sub-



FIG. 142.—(Author.) Sketched from frog's cisterna lymphatica magna, drawn from its edge. (Oc. 2, obj. 8a, Reichert.) It illustrates a group of cells 8, surrounding a stomata verum. 2, 2, 2, the nuclei of the germinating cells of the stomata. 1, points to the three germinating cells of this stomata. 5, 5, shows where there is a rift between the endothelia and the germinating protoplasm, i. e., the soft granulating protoplasm constituting the stomata verum retracts on the application of  $\frac{1}{2}$  per cent. Ag.  $\text{NO}_3$ . 3, 3, indicates nuclei. 6 is an endothelial cell shown not browned by the Ag.  $\text{NO}_3$ . Close observation with high power indicates that the germination in the stomata verum is occurring chiefly under the endothelia, and it shows its dark, granular appearance by shimmering through the old endothelial plates.

peritoneal tissue is the type of all tissue to demonstrate the systems of tubular transportable fluids and the system of interstitial spaces. However, we may observe more gross appearances of interstitial spaces (even lacunae) in the amphibia. It appears from my investigations that in the subperitoneal tissue the blood vascular capillaries are entirely closed from the interstitial spaces except by the so-called stomata and stigmata, while the lymph vascular capillaries are in direct, open connection with the interstitial spaces. This view would show the interstitial spaces of the subperitoneal tissue in more direct and intimate connection with the lymph vascular capillaries than it would with the blood

vascular capillaries. With the great difference in function and structure between the transporting tubular system and the system of interstitial spaces it would not be strange if they were considered not only quite separate but independent systems.

A comparison of the two transporting tubular systems and the interstitial spaces may be instituted by likening them to large swamp land or cranberry marshes. A large, strong river stream (the blood vascular capillaries) passes near the big swamps or marshes which send out small but vigorous streams into the swamp. The fluid collects and slowly moves about in the marsh. The second river (the lymph vascular capillary) collects the fluid from the marsh and carries it back to the original larger river after a longer or shorter time. Vast assimilation and growth may occur in the marsh (i.e., the interstitial space) before the fluids are again collected and transported into the larger river (i.e., the blood stream). Doubtless the reason that the interstitial spaces and the lymphatic ducts have been considered one and the same, without independent distinction, is because the fluid in the interstitial spaces and that in the lymph ducts appear similar. They have some common features, but this comparison is poor in fact as the fluid in the interstitial spaces of the subperitoneal tissue is very different from that in the lymph ducts. The fluid in the interstitial spaces consists of (a) the fluid transported from the blood vascular capillaries; (b) the same fluid plus the effete material from adjacent cell-life and (c) the same fluid minus the material required for cell nourishment. For this reason the lymph flowing in the thoracic duct is a mixture of fluids different from that contained in the subperitoneal interstitial spaces. When the fluid is transuded from the blood capillaries into the interstitial spaces it begins its work at once, which is that of nourishment. The interstitial fluid must give up the needed products of cell-life and must float or drain away the effete matter arising from living cells. In a reasonable sense, then, the interstitial spaces of the subperitoneal tissue may be considered as the field of nourishment, while the lymphatic vessels are mere channels of transportation of fluid. The nitrate of silver aids in making out the lymph channels and interstitial spaces in the subperitoneal tissue by coloring only the solid tissue. It does not color lymph or blood channels or spaces.

Varying conditions arise according as the silver-stained subperitoneal tissue be collapsed or distended by fluid. The mesh-work possesses a wide range of elasticity, and when highly oedematous tissue is well stained with silver it presents a striking feature as to its interstitial spaces. The interstitial fluid and the lymph in the ducts must not be considered identical. It must be remembered that much of the fluid transuded from the blood capillaries passes out by means

of the glandular secretion, in which process the lymphatics are little concerned. We note secretions by the (a) alimentary tract; (b) the respiratory tract; (c) the mammary glands; (d) the tears; (e) the sweat; (f) the salivary glands and (g) testicular glands. Doubtless all these secretions taken together are greater in quantity than flows through the thoracic duct.

An important clinical fact may be gleaned from the absorption of



FIG. 143.—(Author.) A young dog's mesentery. (Oc. 4, obj. 3, R.) 1, 1, 1, endothelial covering of lymph vessels, and light in color. 2, 2, 2, well stained (Ag.  $\text{NO}_3$ ). Endothelium almost like new or germinating, perhaps growing from the open lymph sinus. 2, 2, germinating endothelium around lymph vessels of various shape and very brown. (B) Dog's small intestine peritoneal endothelium. Note elongated endothelia.

fluid from the peritoneal cavity. In extreme and sudden loss of blood, large quantities of fluid could be injected into the peritoneal cavity to restore the volume of blood.

To demonstrate the lymphatics of the diaphragm the rabbit is the most convenient; the cat, dog, guinea-pig, rat, etc., are also available. The rabbit should be killed by bleeding. The abdomen should be opened from pubis to xiphoid appendix. The pleurae should also be opened by snipping into them with a sharp pair of scissors on each side of the breast-bone above the diaphragm. With a toothpick, on which is wound some cotton, very gently brush both the pleural and diaphragmatic surfaces, say two to four times. The brushing must be done rather too lightly than too vigorously. The  $\frac{1}{4}$  per cent. Ag.  $\text{NO}_3$  should be poured on while the diaphragm is in situ. In three to five minutes it can be cut out with the sharp scissors, avoiding all possible dragging. The portion of the diaphragm used for microscopical pur-



poses is the central tendon or centrum tendineum. If it is desired to use the centrum tendineum for other methods of staining it is wise to use the  $\text{Ag. NO}_3$  only for a minute, as the endothelia may be resilvered if very lightly silver stained. A heavy silvering destroys second silvering and makes imperfect ground for other stains. Place the cut piece of diaphragm in distilled water for several hours in the sunlight. To check the silvering add  $\text{Na. Cl.}$  To examine the central tendon simply snip off small bits with sharp scissors and mount in glycerine. The most typical lymphatics, interstitial spaces and channels may be easily observed in the pleural side, with beautiful irregular valved trunks and sinuous endothelia covering irregularly shaped interstitial spaces and lymph sinuses. The most certain location in the central tendon of the lymphatic channels is along the circumference or at the junction of the tendon and muscle. The lymphatics of the central tendon may be seen without brushing, but not as well. The lymphatics of the peritoneal side of the diaphragm are not so numerous nor so easy to demonstrate. The beginner may expect more failures than successes in demonstrating the lymphatics of the peritoneum. The radiating intertendinous lymph spaces are prominent features in the microscopic specimens. To preserve the central tendon place it in 5 per cent. solution of formalin or 75 per cent. solution of alcohol; these agents contract the tissues, however. To demonstrate the diaphragmatic lymphatics with Berlin blue, inject a solution of the colored granules in the rabbit's abdomen and, if killed in forty-five minutes, by brushing and silvering the radiating blue streak may be seen between the tendon and the particles of Berlin blue deposited in all the lymphatics of the diaphragm. The lymphatic trunks of the diaphragm are wide vessels, whose walls consist of a single layer of sinuous endothelial plates. They possess many valves, large sacculations and dilations corresponding to the valves. The posterior portion of the diaphragm collects into two large trunks which empty into the thoracic duct just above the diaphragm. The anterior portion collects into two trunks which accompany the internal mammary arteries. The sinuous endothelia of the many and wide interstitial spaces are easily seen. There are several planes of lymph vessels in the diaphragm united by vertical lymphatic channels. The nuclei of the endothelia may be stained with haematoxylin. The lymphatics may run in company with blood vessels or separate, but as a rule lymphatics course parallel with blood vessels and may be double, lying on each side of the blood vessel. The blood vessels may even be invaginated in the lymph vessels which, however, is nowhere so evident as in the turtle (amphibia). The most distinct lymphatics of the peritoneum, either lymph trunks (with valves) or interstitial spaces, are found in the centrum tendineum. The endothelia of the lymphatics of

the central tendon is chiefly of two shapes, (a) elongated spindle-shape and (b) sinuous bordered. The endothelia are elongated in the direction of the lymph stream just as blood vessels have elongated endothelia in the direction of the stream. At the valves, duplicatures of the intima, the endothelia changes its direction to transverse, chiefly. In the lymphatics swim a number of round bodies, lymph corpuscles. In some vessels they are very numerous.

THE AMPHIBIAN LYMPH SACS. (CISTERNÆ LYMPHATICÆ MAGNÆ AMPHIBIÆ.)

Situated on each side of the dorsal part of the abdomen of amphibia are two large lymph sacs. They are about the size of the first joint of the little finger, in adult frogs, and proportionately large in turtles. They are situated just below the liver or just posterior to the usual site

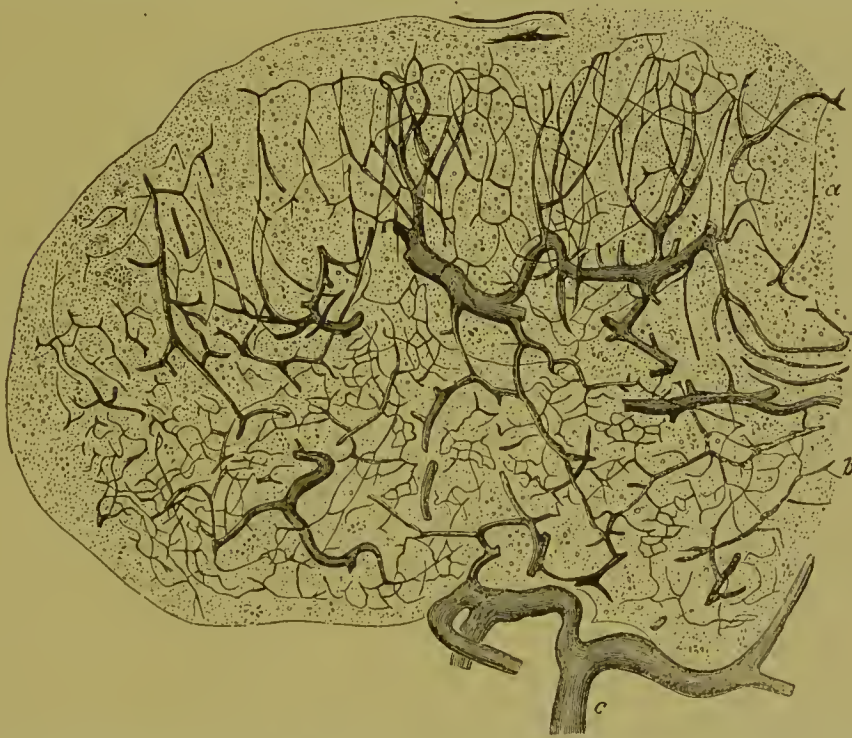


FIG. 144.—(Handbook for Phys. Lab., Vol. II., 1873.) Vertical section of injected mesenteric gland of guinea-pig, showing the distribution of the blood vessels. (a) Cortical layer. (b) Medullary layer. (c) Large blood vessels of the hilus of the gland. (Oc. 3, obj. 2.)

of the diaphragm (as frogs have no diaphragm). The lymph sacs are covered by peritoneum. On the peritoneum of these lymph sacs are located peculiar structures known as stomata, first described, so far as I am aware, by F. Schweigger-Seidel and J. Dogiel in 1867, from the laboratory of Karl Ludwig in Leipsic. Panizza and others long before described the lymph sacs. B. Mascagni had asserted long ago that the lymph vessels stand in immediate connection with the serous cavities of the body. Schweigger-Seidel and Dogiel claimed (1867) that the peritoneal cavity is only a dilation of a lymph passage. The typical

stomata of the whole peritoneum, as regards exact structure, are formed in the peritoneal covering of the amphibian lymph sacs. The wall of the lymph sacs consists of a connective tissue layer and two endothelial layers. One endothelial layer belongs to the peritoneum and the other endothelial layer belongs to the lining of the lymph sacs. If a communication exists between the peritoneal cavity and the lymph sacs, the three layers must be so arranged that the stomatal openings should correspond with openings in the ground or connective tissue layer.

The ground or connective tissue layer of the sac consists of bundles of connective tissue sparsely provided with elastic fibres. Schweigger-Seidel and Dogiel claim that the ground layer of the lymph sac contains perforations which exist between the bundles of connective tissue. The holes or perforations correspond to the stomatal openings of the endothelial layers applied on each side of it. These perforations are then exactly similar to the perforations existing in the *membrana limitans* located on the diaphragm and discovered by Bizzozero, 1874. The apertures are oval, round or slit-like. The size and distribution of the stomata are very various as well as the perforations in the ground layer. The stomata are, however, numerous on the peritoneal side of the lymph sac. Of course, all kinds of openings in the lymph sac wall vary according to the degree of stretching to which it is subject. Under low power the lymph sac wall looks like a sieve, but under high power the distinctly organized stomata or mouths may be observed as lined with granular, polyhedral nucleated protoplasmic cells which stain highly on the application of  $\text{Ag. NO}_3$ . The apertures in the ground layer corresponding to the perforations of the *membrana limitans* are quite numerous. The endothelial cells of the peritoneum covering the lymph sac are large, irregular and somewhat elongated, which is doubtless due to the expanding and contracting of the sac. The endothelial cells converge or radiate about a stomata, corresponding no doubt to a preformed opening or a primordial arrangement. Schweigger-Seidel and Dogiel claim that such a preformed opening or stomata corresponds exactly to an opening in the ground layer of the lymph sac.

In one respect I am convinced that the above authors are wrong in concluding that the cells surrounding the stomata are the nuclei of the adjacent endothelial plates. It is claimed that endothelial borders show better if the serous membrane be dipped in hot water first, and also that the carmine will more vigorously color the nuclei. The shape of the endothelia lining the lymph sac is irregularly polyhedral. Stomata in the endothelial layer of the lymph sac are noted to correspond with the apertures in the ground layer and peritoneal covering of the sac. It appears that the stomata in the lining of the lymph sac are smaller than those of the peritoneal side; however, perhaps that depends on the



degree of straining of the membrane. The degree of straining the lymph sac by breathing and muscular action give a physiologic significance to the stomata and apertures of the sac wall. As the stomata and apertures open and shut the fluid passes and repasses according to pressure. Muscular play aids the lymph sac to functionate by contraction and dilation of the stomata and apertures.

If fluid containing colored granules be injected into the amphibian peritoneum the colored material rapidly finds its way into the lymph sacs. Panizza claimed that the whole lymph system is connected while

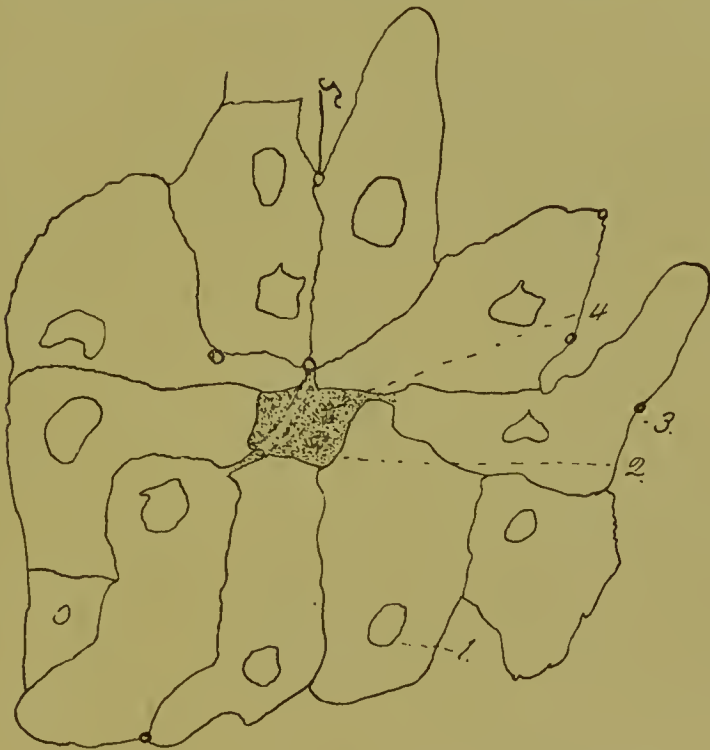


FIG. 145.—(Author.) Is drawn from the lymphatic cisterna magna of a frog; 1 shows the nucleus; 2 points to a stomata verum, and 4 points to the nucleus of the cell lining the stomata; 3 indicates the endothelium; 5 points to a stomaspurium. Drawn under very high power. Note the grouping of 9 endothelia around the stomata verum, 2.

Jos. Meyer, in a work entitled "Systema Amphiborum Lymphaticum," Berlin, 1845, combated Panizza's views by noting that the reason injected material (or air) found its way into all tissues of the lymph is because the force of the injection tore or ruptured it. Meyer is undoubtedly in error, for a physiologic absorption carries the colored granules into the lymph sac.

Germinating endothelia or ciliated endothelia have attracted considerable attention from investigators of the peritoneum. The reason that germinating endothelia are spoken of in this place is because in the region of the germinating endothelia may frequently be found a

lively and rich production of developing lymphatics either on a lymph sac or on other parts of the peritoneum, especially in the frog.

In Professor Ludwig's laboratory at Leipsic began the study of the



FIG. 146.—(After Handbook for Phys. Lab., Vol. II., 1873.) Lymphatics of centrum tendineum of rabbit, pencilled under water and then bathed in silver, while artificial respiration was being carried on. The lymph vessels are visible in the slightly colored ground as distinct and very sinuous tubes, the endothelium of which is sharply defined. (a) Trunks of lymph vessels of pleural side (b) Lymph capillaries, which as "straight interfascicular lymph capillaries" run between the tendon bundles, and reach to the abdominal side. (Oc. 3, obj. 5.) Observe that sometimes the lymph vessels become sharply narrowed. This is at places where they are compelled to pass between narrower spaces, a cleft between tendon bundles.

so-called germinating endothelia of the peritoneum. The Germans call it "flimmerepithel" (endothel). However, the keen old investigator, Leydig, in his often quoted book on histology (1858) speaks of the glittering germinal or ciliated endothelia in frogs. It is thought by those

who advocate this view that the whole endothelia of the peritoneum is ciliated or possess short cilia. Also another class of older investigators, believe that the cilia are located at the circumference of the stomata



FIG. 147.—(Author.) Human ligamentum suspensorium hepatis. It was pencilled or brushed and then stained with Ag.  $\text{NO}_3$ . These endothelia likely cover lymph channels. 1, stoma verum; 2, nucleus of germinating cell; 3, stoma verum; 4, stomata spuria.

and that the motion of the cilia forces the current onward as it does in the epithelia of the Fallopian tube.

The germinal endothelia are considered smaller than the general endothelia. In various portions of frog's peritoneum the germinal endo-



thelia form an ornamental kind of mosaic. They are seldom ever found alone or single, but radiate and form a network of endothelia smaller than the adjacent normal ones, and they stain highly under silver. The new growing endothelia stand in groups, cords, strands or coalesced

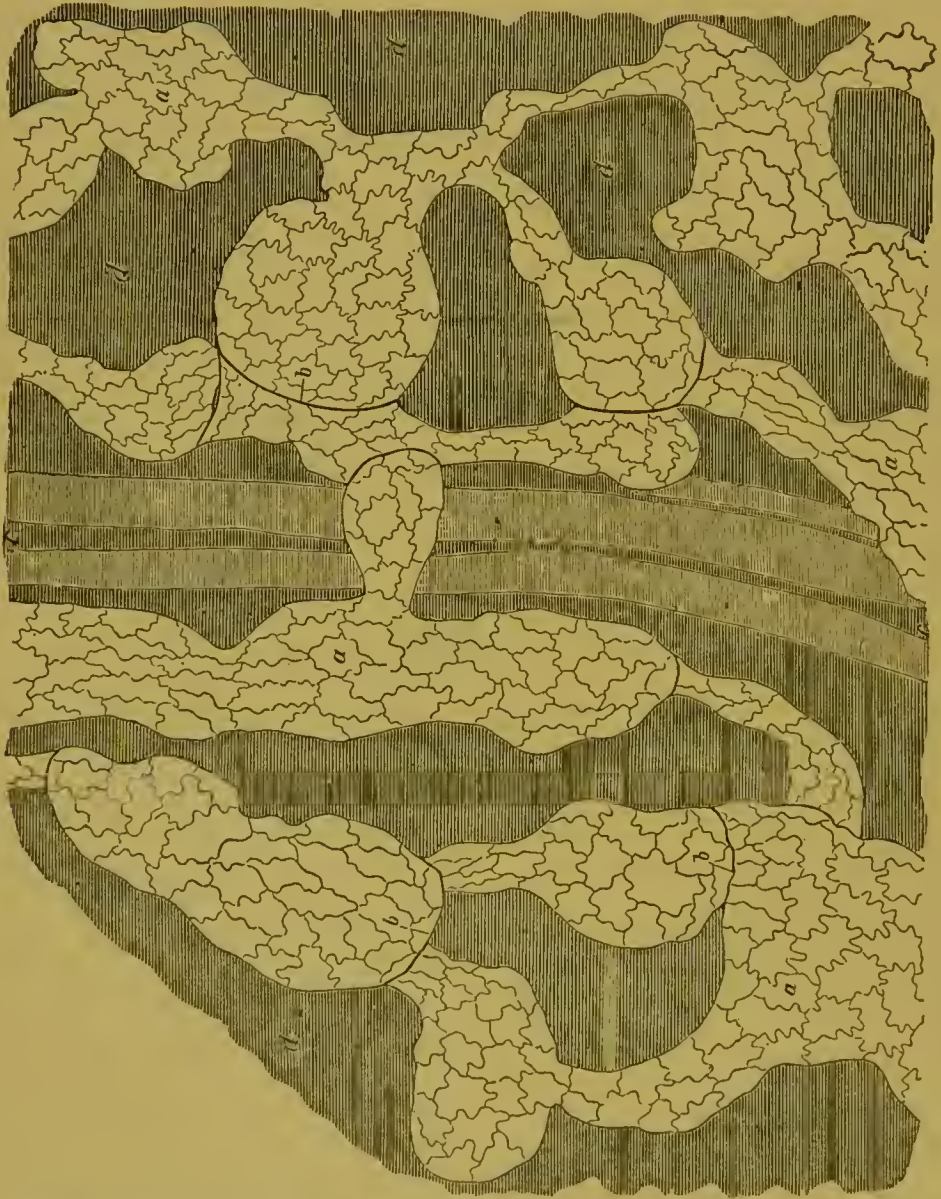


FIG. 148.—(After Handbook of Phys. Lab., Vol. II., 1873.) Surface of omentum of rabbit pencilled and colored in silver, showing the distribution of the lymph vessels. (a) Lymph vessels, showing their endothelium. (b) Valves. (c) Indicates the position of vessels enclosed in a tract, the details of which, as well as those of the ground substance (d) are omitted. This is a rare and excellent specimen, as it is not always easy to demonstrate such beautiful lymph vessels in the rabbit's omentum.

into large patches. The ciliated endothelial cells are characteristic, especially in pregnant female frogs and turtles, and in them are located on the mesentery of the ova-sac or when the long ova-sac frictionizes against the peritoneal wall. Increased friction and increased blood

supply doubtless play a role. Perhaps the germinal ciliated cells are creatures of transitional periods existing during the pregnant season of the amphibians.

Schweigger-Seidel's view that the ciliated, germinal, granular cells around the mouth of a stomata are the nuclei of the adjacent cells, I think, must be abandoned. Again, he advocates that the cilia at the circumference of the stomata are instruments to propel fluids might well be answered that cilia also check fluid currents. It may be stated that the walls of the amphibian lymph sac are very sparsely supplied with blood vessels. But it is the typical place to demonstrate the peritoneal nerves by the aid of Ag. NO<sub>3</sub> or Au. Cl<sub>3</sub> (1 part), plus C<sub>2</sub> H<sub>4</sub> O<sub>2</sub> (5 parts), plus H<sub>2</sub> O (996 parts).

The peritoneum is a lymph sac. It is lined by a single layer of peritoneal endothelia. The endothelia in some places lie on the lymph spaces with but little intervening tissue between them and the endothelia of the lymphatics. In other places the endothelia do not have lymphatic spaces immediately under them. The peritoneal cavity is in direct connection with the walls of the lymph sac, so far as I have made examination of the peritoneum. The order of localities where lymphatics highly abound are: 1, the tendinous portion of the diaphragm; 2, the ligamenta lata; 3, the omentum; 4, the ventral surface of the small intestines; 5, the liver and spleen. The lymphatics might be considered the drainage of the peritoneum. The lymph channels of the peritoneum stand in open connection with the peritoneal cavity by means of vertical canals lined with granular cells. The lymph channels of the peritoneum are viewed with the microscope in two ways, viz.: (a) after staining the free peritoneal surface with Ag. NO<sub>3</sub>, a small bit is snipped off, mounted in glycerine, and the lymphatics viewed by looking through the transparent endothelia of the free peritoneal serosa. Vast lymph channels and wide tracts of capillary lymphatic vessels may be seen, especially in the zona tendinea of the diaphragm. (b) By brushing off the peritoneal endothelia and then staining with Ag. NO<sub>3</sub> and mounting in glycerine, one can view the lymph vessels and capillaries directly. Another method might be mentioned, which is to inject the lymph vessels either by a physiologic or mechanical method.

The location of the lymphatics of the peritoneum in general may be placed immediately beneath the free peritoneal serosa. The microscope may reveal one or several layers of lymph vessels.

The signification of the lymphatics of the peritoneum is that of nutrition and drainage. The lymph fluid passes out of the blood vessels into the spaces of the sub-endothelial tissue in which are found the lymph channels. The channels, after the tissue has been bathed by



the lymph fluid, conduct it back to the blood vessels, finally the sub-clavian veins.

The origin of the lymphatics of the peritoneum lies within the subserous connective tissue. In the subserous tissue may be seen cells with branching stellate cells, with various kinds of projections. The branched or projecting processes of these cells communicate with or join each other in such a manner as to form tubes or channels through which flows the fluid lymph. Also in these spaces are found the lymphoid



FIG. 149.—(Handbook for Phys. Lab., Vol. II., 1873.) Bud-shaped structure of mesogastrium of frog, treated with silver, covered with ciliated, polyhedral germinating endothelium. In the ground substance of the bud-shaped structure are groups of young amoeboid cells; and in addition to these are vacuole cells beset with cilia on their internal surfaces, i. e., that turned toward the cavity of the vacuole. There is also a large vacuole cell, the wall of which has become changed into endothelium. (Oc. 3, obj. 8.) One can find ciliated cells in the frog's and turtle's peritoneum, especially during gestation.

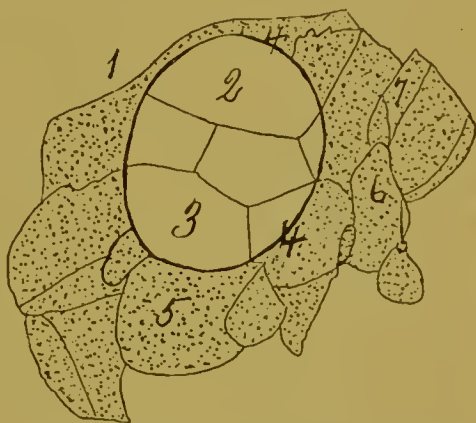


FIG. 150.—(Author.) Human omentum majus. (Oc. 4, obj. 3.) A vacuolated cell taken from a point where only one existed. 1, edge of trabecula; 2, 3, endothelia on vacuolated cell. Really 2 and 3 are the bottom cells of a stoma verum. The stoma is well defined on its circumference, 4, 4. This will develop into a lymph sinus or lymph vessel by constant widening or vacuolating. Observe that the endothelia at the bottom of the stoma is in contact with the germinal of lymph endothelia adjacent to it. 5, 6, 7, are germinal and lymph endothelia. They are very brown from application of Ag. NO<sub>3</sub>. Immediately adjacent to this drawing are large spaces showing beautiful vacuolated cells in all stages of development—soon to become lymph sinus and vessels. The vacuolated cell wall is differentiated with endothelial plates.

cells. The spaces thus formed are the so-called “juice canals, saftkanalchen,” of Von Recklinghausen, or what medical literature terms the lymphatic canalicular system. In this lymph canalicular system lies the fixed connective tissue corpuscle and the wandering or lymph corpuscle. The fixed connective tissue corpuscle remains permanently in its location, but the wandering corpuscle is simply a blood (white) corpuscle which has passed out of the blood vessels and entered the lymph channels. The lymph canalicular system communicates with the so-called



lymph capillaries, and in good specimens one can plainly observe the transition from the lymph capillaries into the lymph channels. The peritoneal lymph capillary is easily distinguished by being lined by endothelia with a very sinuous margin. The lymph capillaries are simply larger than blood capillaries. The lymph channel, which collects the fluid, generally lies in the middle of the capillary space which coalesces toward the channel. The means by which the blood reaches the capillary lymph space is not fully settled. It is assumed that there are temporary or permanent openings in the walls of the blood vessels. These openings we have so far in this work designated stomata spuria and vera. J. Arnold designates the larger apertures in the blood vessel walls as stomata and smaller ones stigmata.

The stomata (vera or spuria) occur at the common junction of several of the endothelia which compose the wall or along the single inter-endothelial line. The fluid which passes out of the blood vessels into the tissue nourishes it, i. e., the tissue appropriates the part of the fluid required for its assimilation and the effete matter passes on in the lymph stream, through the lymph capillaries, lymph channels, through the lymph glands, through the thoracic duct to the subclavian veins. The method of propelling the lymph fluid is a matter not yet fully decided, but blood pressure and muscular action have much to do with it. The connective tissue corpuscles may aid by relaxing and imbibing the fluid and then contracting and expelling it, thus inducing a movement in the stream. The lymph corpuscles, leucocytes, we know pass onward from the blood vessels through the lymph capillaries, channels, glands and finally through the thoracic duct, because by impregnating them with coloring matter, they pass to various portions of the body. Leucocytes wander through the lymph spaces and pass into the lymph channels. For example, experiments show that finely divided coloring matter held in liquid suspension injected into the peritoneal cavity is quickly and first found in the vast lymphatics of the diaphragm. From considerable observation and experiments to show that white blood corpuscles (leucocytes) will pass through the stomata of blood vessels and blood capillaries as a normal physiologic process, it is natural to assume that cellular elements may pass from blood vessels and capillaries into the spaces or origin of lymph capillaries as a normal physiologic process. Granular coloring material will pass from the blood into the cells within the lymph spaces. If the granular coloring matter is excessive it will accumulate in the lymph canalicular spaces. Again, there may be considered the subject of peri-vascular origin of the lymph vessels. It is easily demonstrated that the turtle has especially typical peri-vascular lymph spaces. The blood vessels in this case lie inside of a large lymphatic tube. I have found the same peri-vascular conditions in the

frog's mesentery. The space extending between the external wall of the blood vessel and the surrounding lymphatic tube is known as the



FIG. 151.—(Author.) Drawn from frog's mesentery (oc. 2, ob. 8a. R). Ag.  $\text{NO}_3 \frac{1}{2}$  per cent. applied until the common surface endothelium was very brown. 1 and 2, blood corpuscles lying on the endothelial cell. 3, cleft in endothelium corresponding mainly with the underlying lymph endothelia. 4, 4, remnant of endothelial cells; the other parts were pencilled away. 5, 5, a remnant of a stoma verum; 6, a stoma verum of the underlying lymph endothelia covering lymph sinuses or spaces. 7, an enormously long lymph endothelial cell; 8, another long lymph endothelia. 9, 9, shows very distinctly the broken edge of the common surface endothelium. I had in this specimen a good opportunity to observe the elasticity of the common surface endothelia at this broken marginal line. The endothelia would assume a curled, bent shape, and by making waves of water bend the curled cells straight they would spring back to their curled condition as soon as the waves passed over. The common surface endothelia, 4, 4, was very brown. 10, 10, nuclei of endothelia covering lymph spaces. 11 and 12 show two nuclei; 13, nucleus of common surface endothelium. Note the difference between the very brown, large irregular endothelia of the common surface and the narrow elongated light-colored endothelia covering lymph spaces with very small amount of intervening substance.

peri-vascular space of His. The space is divided into departments by radiating partitions. In the peritoneum where the peri-vascular spaces

accompany blood vessels, the passage of blood and lymph corpuscles is highly facilitated. Gegenbaur claims that the peri-vascular spaces in the turtle are visible to the naked eye. But the peri-vascular spaces of the peritoneum of all animals which I have examined from the frog upwards are microscopic, and besides are neither typical nor easy to make out in every case. I have examined several turtles, and the peritoneum shows typical lymphatic lacunae, wide interstitial and peri-vascular spaces.

In my remarks on peri-vascular spaces in the peritoneum I do not include the islands of tissue found in large lymph channels. These islands, called by Klein endo-lymphangial spaces, are entirely surrounded by the fluid in the lymph channels. I have found typical conditions of endo-lymphangial spaces on the pleural surface of the diaphragm which has been well pencilled so as to desquamate its endothelial covering. The old views of Mascagni represent well the modern views that permanent structures, stomata, vertical channels or openings which could be temporarily formed, produce an open, direct communication between the peritoneum and the lymphatics. The names which lend their weight in favor of a free communication between the peritoneum and lymph spaces are Ludwig, Schweigger-Seidel, Klein, Recklinghausen, Dogiel, Dybkowsky and Notkins. Against these views may be observed such names as Frey, Muscatello, Kolossow, Auerbach and Affannasiew. The non-agreement is not that such openings (stomata) do not exist, but one series of investigators claims that stomata permanently exist and have fixation, structure and location, while the others claim that stomata exist only where blood corpuscles or leucocytes by pressure produce a widening or opening in the interendothelial space which can and will close down again after the corpuscles have made their exit. With them the stomata have no fixation, structure or definite location. With them the stomata arise and disappear as necessity demands, i.e., according to the conditions of circulation. All admit that fluid placed in the peritoneum passes into the lymphatics immediately below the peritoneal endothelia. The common ground of all investigators in the peritoneum may be considered to be the large lymph sacs located in the abdomen of the frog. In this lymph sac, *cisterna lymphatica magna* are plainly seen with the microscope, small apertures, stomata around whose mouth are located large, irregular polyhedral cells, known as germinating cells. The stomatal, germinating cells undergo a change in shape so that the stomata present an open, partially closed, or closed condition. The cells at the mouth of the aperture contract and expand. These stomata are the origin of the lymphatics, and on these positively demonstrated structures rest the common views of microscopists. Schweigger-Seidel claims also that the *membrana limitans*



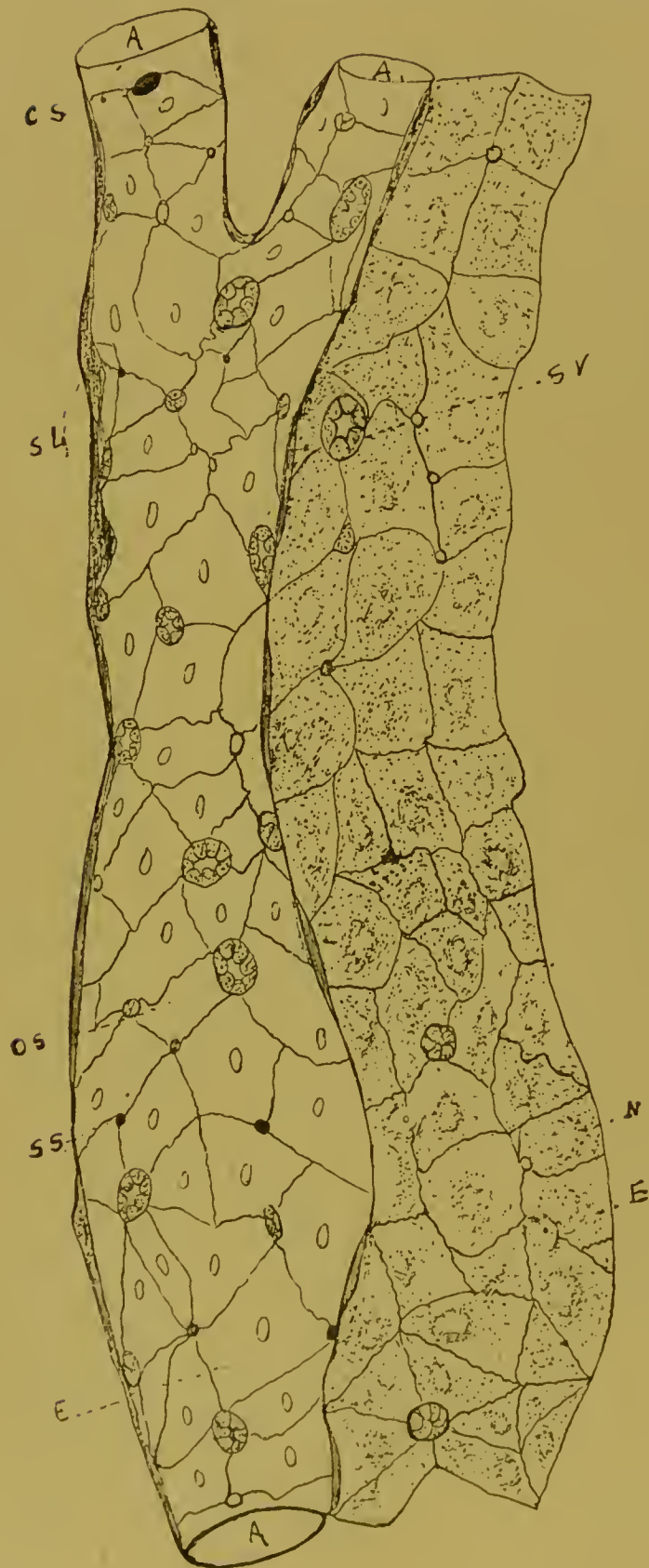


FIG. 152.—(Author.) Drawn from the pyloric end of a rabbit's omentum. The omentum has been pencilled, stained with silver and logwood. A, represents a lymph channel covered only by lymph vascular endothelium which alone

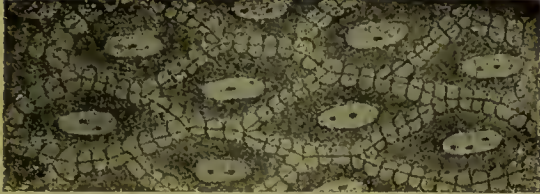


FIG. 153.—(A. Kolossow, 1895.) Endothelia from a vein of a dog, showing the anastomotic connection of the cells.

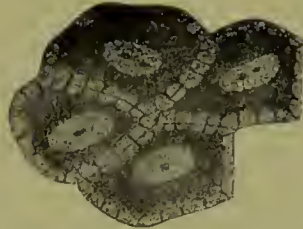


FIG. 154.—(A. Kolossow, 1895.) Endothelial cells from the peritoneum of the duodenum of a dove, showing nuclei with nucleoli and anastomotic processes binding the cells together.

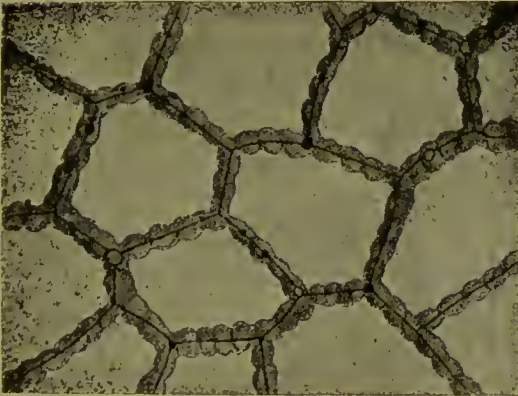


FIG. 155.—(A. Kolossow, 1895.) Represents the interendothelial canals filled, from the stomach of a frog. Black dots and rings appear in the interendothelial plates. The irregular, bead-like bulgings noted in the interendothelial space are considered to be the spaces between the anastomotic processes filled out with some colored material. The specimen was treated with  $\text{Ag. NO}_3$ , osmic acid and tannin.



FIG. 156.—(A. Kolossow, 1895.) Endothelia from the frog's mesentery, showing oval nuclei, anastomotic processes, treated with  $\text{Ag. NO}_3$ , osmic acid and tannin.





encloses the wall of the lymph vessel. The peritoneal endothelium has been brushed away. The peritoneal endothelium (E) with its nucleus (N) shows a very different appearance than lymph vessel endothelium. Observe that the endothelium covering the lymph channel is not only sinuous but quite irregular in outline. It does not take on the coarse, granular appearance of the peritoneal endothelium by the use of silver salts. Notice how the wall of the lymphatic channels shows its nucleus (S. L.) in profile very distinctly. The nuclei (N) show very plainly after staining with logwood. They are very frequently situated to one side of the center of the endothelial cell. Again, observe little, open-mouthed channels stomata vera (S. V.). They are open (O. S.) stomata or closed as at (C. S.). There occur also small black dots as at S. S., known as stomata spuria. The stomata spuria are located at the common junction of several endothelial cells shown by the silver lines. They are described especially by Oedmansson, and most histologists consider them as communications between the peritoneal cavity and the underlying lymph channels. Some think they are the projections of a connective tissue cell coming to the surface to repair or to take the place of the degenerated endothelia. The microscope does not with silver salts, it seems to me, indicate very definitely what these so-called stomata spuria (S. S.) are, but the stomata vera show especially plain in this figure. Some are closed C. S., while others are open, as O. S. The stomata vera (S. V.) are lined by polyhedral cells of a more or less granular character. In this figure they are very numerous and stand in irregular relation to each other. This open and shut condition of the stomata vera may account for the sinuous lines of the lymphatic channels lying so irregular. The stomata vera are vertical lymph channels which produce direct communication between the peritoneal cavity and the subserous lymph channels. Injecting milk or Berlin blue into the abdominal cavity is followed by the material passing into these stomata vera. The study of the peritoneal lymphatics through the stomata vera and spuria will be followed by knowledge in the right direction, for clinical knowledge of the peritoneum is in the lead of physiologic and pathologic knowledge.

under the peritoneum is perforated. But as to the investigations, wider divergent views are obtained from modifications of structure and additional complications of methods of investigation. The peritoneum must then be considered a true lymphatic space and the fluid it contains lymph fluid. Also it contains channels, stomata vertical canals which produce free, open, direct communication between it and the sub-endothelia lymphatics or space.

The peritoneum is a lymph cleft produced by fluid pressure and independent motion of viscera and body wall, and hence the walls of the peritoneum will contain the high supply of lymphatics belonging to all mesoblastic tissue adjacent to viscera. Since the peritoneum itself is a lymph space or cleft, of course its walls must necessarily be lymph walls, i. e., containing lymph spaces or vessels. The large lymph trunks resemble veins; however, they possess many more valves. The lymph spaces in the peritoneal wall may be viewed by the microscope as (a) capillary lymphatic fields, known by the large, sinuous, irregular endothelial plates, (b) distinct lymph channels possessing definite walls and dilations and contractions of the vessel lumen, and (c) as large, wide lymph channels known by the quite uniform spindle form or elongated endothelia. I think I have seen such lymph endothelia twelve times as long as broad.

In this work we are considering a lymph sac, the peritoneum, endowed with the function of secretion and absorption of fluid. It always

controls a certain amount of fluid in the sac, and hence the object will be to interpret how and by what means the peritoneum becomes an automatic lymph sac. For by the discovery of anatomic structure and physiologic function prophylactic measures against disease are made possible. The lymphatic vessels of the peritoneum are bounded by distinctly separate and independent walls. They are not merely spaces in the connective tissue without boundary walls, but independent anatomic channels.

Considered from a physiologic view as a result of the blood pressure



FIG. 157.—(Author.) Drawn from the lymphatic capillaries of a wood-pecker's peritoneum under 1-15 oil immersion lens. Ag.  $\text{NO}_3$ ,  $\frac{1}{2}$  percent. The bird's peritoneum has very wide interendothelial spaces. There are many more rings and transverse anastomotic processes than I have drawn. I drew the prominent ones. The endothelia of the lymph capillaries 1, 2, 3, are proportionally longer than represented in the figure. Often one endothelium reaches far beyond the microscopic field. 4, 5, 6, doubtless represent stomata vera. All the nuclei are not sketched. This specimen illustrates beautifully the interendothelial space and that the hypothetical cement substance should be discarded. Also that the interendothelial space is the same in the lymphatics and peritoneum, i. e., it contains stomata vera and spuria. 5 and 6 represent double nuclei.



FIG. 158.—(Author.) Drawn from frog's cisterna lymphatica magna. (Oc. 2, ob. 8a, Reichert.) The surface directed toward the cisterna is shown. Eight stomata vera are shown; some partially open, others tightly closed. Some of the cells of the stomata vera are marked with nuclei. There are scores of other stomata vera adjacent and distributed similarly. 2, the stomata vera cells showing no nuclei; 3, with two nuclei; 4, with one nucleus; 5, an elongated stomata verum.

in the vessels of the wall of the peritoneum, the subserous tissue is being constantly bathed with fluid—lymph. This lymph fluid nourishes the tissues, it bathes the connective tissue cells with a liquid medium rich in any element required for assimilation. Again, the lymph floats away all effete matter, all waste-laden fluid moves on in the continual stream. Besides, the lymph is a fluid medium in which can functionate the contraction and relaxation of the connective tissue cell, which no doubt requires a fluid medium to carry on its life, its waste and repair. The lymph stream doubtless depends much on the blood pressure and muscular action for its existence and activity. The lymphatic

system of the peritoneum is an appendage of, and dependent upon, the blood vascular system. In fact, blood vessels and lymph vessels exist only together. Hence the vigor of the blood stream will determine that of the lymph stream. In health, then, there must be an active blood and lymph stream, and one depends on the other. If the lymph stream of the subperitoneal tissue did not produce a perfect drain of the effete material, waste-laden fluid, the lymph stream would not only be clogged itself, but the blood stream would also suffer an obstruction. The lymph canalicular system of the peritoneal tissue forms a peculiar system



FIG. 159.—(Stohr, 1894.) Represents a lymph vessel with valves drawn from the mesentery of a rabbit. Magnified fifty times. The endothelia are well drawn.



FIG. 160.—(Author.) Lymph vessels of pleural surface of diaphragm of rabbit. Pencilled and Ag.  $\text{NO}_3$   $\frac{1}{2}$  per cent applied. 1, 1, valves; 2, 2, 2, 2, lymph capillaries with endothelia delineated; 3, 3, 3, lymph vessel trunks whose endothelium is not marked. Note the irregular bulging of the walls; 4, 4, 4, ground substance not drawn. (Oc. 4, ob. 3, R.) 6 and 7, valves of capillaries opening into the main trunks.

whose meshes are so scattered that it indirectly receives the lymph fluid from the blood vessels and finally returns the same lymph fluid, plus the effete matter, i. e., the wear of the living subperitoneal tissue, to the blood vessel. The rapidity of the lymph stream does not depend so much upon the vigor of the stream as on the absolute difference in pressure between the blood vascular and lymph vascular systems. The lymphatic system is an acquisition of vertebrate life, a necessary apparatus to nourish tissue distant from blood vessels. It is a drainage apparatus, a second vascular system. It may be noted that the subperitoneal tissue, which is highly supplied with blood, is highly supplied also with lymphatics, and yet there are organs which are especially adapted for absorption, as the diaphragm, which possesses a rich supply of abundant lymph vessels.

There are three questions which seem to have concerned all recent investigators in regard to the lymphatics of the peritoneum: 1, the



distribution of the lymphatic vessels in the peritoneum; 2, the origin of the lymph capillaries from the lymph canalicular system of Recklinghausen and 3, the relation of the connection of the lymphatic system of the subperitoneal tissue to the free peritoneal serosa.

In studying the lymphatic system of the peritoneum microscopically, two subjects are continually in the field. The first is the lymph fluid in the lymph capillaries, i. e., as soon as the lymph escapes from the blood vessels it circulates in the interstitial spaces, in the serous canals or the lymph canalicular system. This section may be called also the lymphatic capillary system. The second part of the lymphatics of the subperitoneal tissue are the definite lymph vessels or lymph channels having defined and outlined borders and valves. We will consider first the lymphatic capillary system. In the capillary system will be included the interstitial serous canals.

We recognize the lymphatic capillaries of the subperitoneal tissue by the irregularity and sinuous character of the endothelial outlines. The endothelia are not only sinuous in outline, but have large loops which sometimes have a small contracted neck. The wavy loop may be directed outward or inward on the edge of the endothelial plate. The lymph capillaries are exceedingly variable in shape, outline and size. They may be very small in size and acutely serrated in outline, or endothelia may be joined and the outline consist of wide-sweeping curves. They are always known by the sinuous character of their outline. I do not mean by this assertion that lymph capillaries of the subperitoneal tissue are not tubular in character. In the frog we may notice a large part of the lymph capillaries as tubular in form. In the human broad ligament, in which I made extensive observations, I found the majority of the superficial lymph capillaries distinctly tubular in outline, and the endothelia forming the lymph capillaries of the ligamentum latum was very much elongated and quite sinuous in outline. It was long spindle-shaped endothelium in which the major diameter occasionally exceeded the minor diameter by at least a dozen times. Very delicate tubular lymphatics can be observed on the pleural side of the human diaphragm, and especially on the diaphragm of the guinea-pig. The subperitoneal tubular lymphatics are characterized by irregularity of lumen, little bulgings and constrictions representing valves. Now it is characteristic so far as I have observed that these bulgings or sacculations occur frequently at points of junction of the capillary vessels, as it is here that the endothelia is apt to become transverse to some extent and produce a so-called valve. The dilations and contractions may succeed each other at short intervals. Yet considerable distance intervenes on same tubular lymphatics without the visible trace of a valve, which no doubt prevents the regurgitation of lymph fluid. A peculiar-

ity I have noted in the superficial lymph capillaries of the human broad ligament is the presence of single patches, islands of very sinuous, irregular lymph capillaries in the midst of wide lymph channels possessing quite uniformly shaped endothelia. The serous lymph canals or lacunae are the beginning or roots of the lymph capillaries. These lacunae, little chains of lakes well express their appearance under high power. These spaces lie in the clefts of the ground substance, and in the channels lie the connective tissue corpuscle, bathed in the lymph fluid. I will describe them from the best specimen which I found in the human diaphragm. The lacunae can be distinctly traced from their source to the lymph capillary, into which they pour their



FIG. 161.—(Author.) Dog's broad ligament. 1, germinal endothelia; 2, lymph endothelia requires a different focus; 3, 3, stomata vera; 4, stomata spuria. (Oc. 2, ob. 8a.) On the side not surrounded by germinal endothelium, the large germinal endothelia extend around the lymph vessels. This broad ligament is a panorama of changes, growth, vacuolation and germinating endothelia.



FIG. 162.—(Author.) From frog's lymphatica cisterna magna, cisternal side. (Oc. 4, ob. 3, R.) Ag.  $\text{NO}_3$ , 2 per cent. The stomata are closed. Fig. 161 shows peritoneal, and Fig. 162 cisternal side, drawn from the same specimen. I first drew (a) and then reversed the slide and sketched (b.) One cannot decide the difference of the stomata on each side in separate specimens. 1 and 2, stomata vera

contents. The lymphatic serous canals or canaliculi are the mediators between the blood vessels and the lymph capillaries. These juice canals have a winding, sinuous course among the cells composing the ground substance, here a constriction, an isthmus, there a widening out of the stream into a lake. But the channels can be distinctly traced from the lymph capillary outward through the clefts on the ground substance. The lymph canalicular system can be easily observed to terminate in the lymph capillary system, in fact, they are directly continuous with each other. In other words, the lymph capillaries take their rise from the lymph canalicular system of Recklinghausen, or what we term interstitial space.

We will now consider the lymph vessels, the efferent canals of the

lymph capillary system. The lymph vessels resemble the blood vessels in possessing three tunics. The large lymphatic vessels are distinctly tubular in character except in the amphibia, of which I have so far only studied the frog and turtle. In the frog, and especially in the turtle, one can observe large, wide spaces or lacunae which are lymph spaces. These places have irregular outlines and are varied in size. If one will observe sections of the pleural side of the diaphragm under the microscope very fine specimens will be observed as regards lymph vessels, and also the anatomical fact may be observed that when blood vessels are abundant lymph vessels are also abundant. In fact, lymph vessels are a direct dependent on blood vessels—vascular apparatus appended to the blood vessels. An especial feature of the lymphatic vessels is a valvular condition. The valves consist of a duplication of intima and in some vessels are quite numerous, especially at the junction of the channels. On the distal side of the valve the lymph vessel is dilated and may be sacculated. It can be observed from this fact that the lymph vessels do not preserve a uniform calibre for long distances. The peculiarity of the lymphatic system has been noted by J. Miller, Von Recklinghausen and others. As regards large lacunae in the subperitoneal tissue, Gegenbaur observes that in the turtle one can find the blood vessels completely sheathed or invaginated in lymph vessels. This fact I have not only observed, but have presented in the work by sketches. The blood vessels are completely surrounded by a lymph vessel, known as a peri-vascular condition; perhaps it would be better to term it circum-vascular. The blood vessel can be seen inside of the lymph vessel like a black rod. The peri-vascular or circum-vascular lymph vessel is divided into spaces or compartments by septa radiating from the blood vessels to the outer wall of the lymph vessel. Those compartments have been called the peri-vascular space by His. Great lymph sacs appear in the frog known as *cisternae lymphaticae magnae*. Perhaps these lymph sacs in the frog supply the place of a diaphragm, as the frog has no diaphragm. The lymph sacs communicate with each other by small openings. One fact may be noted by the allusion to the lymph sacs of the amphibians, and that is the wide variability of the lymphatic system in all animals.

A very important question is, what is the relation of the lymphatic vessels and capillaries to the surrounding tissue? Do the lymph vessels have independent walls or are the walls of the lymph vessels (and capillaries) fused with the adjacent tissue? Studies by injection and the introduction of the method of staining with Ag.  $\text{NO}_3$  by Von Recklinghausen have demonstrated that the finest lymph capillary is composed of an independent, separate and distinct wall. The wall may be simply a single layer of nucleated endothelium so joined edge to edge that a closed



tube or vessel is formed. The wall of the lymph capillary is not a part of the adjacent connective tissue, as was formerly announced, but consists of endothelium. It is important to decide the independent nature



FIG. 163 —(Handbook for Phys. Lab., Vol. II., 1873.) Silver preparation of the septum of the cisterna lymphatica magna in a female frog. (a) Endothelial elements of peritoneal surface having germinating characters. (b) A free trabecula projecting above the surface covered with germinating endothelium. (c.) Pigment cells. Note how irregular in shape and size are the endothelial elements.



FIG. 164. —(Author.) From frog's lymphatica cisterna magna peritoneal side. (Oc. 4, ob. 3, R.) It shows stomata vera mostly closed. 1 is open, the remainder closed. Ag.  $\text{NO}_3$ ,  $\frac{1}{2}$  per cent. (It is very difficult to say positively that some are open, for the space is filled with granular matter which resembles the stomata vera cells. The figure was sketched under good sunlight, as near to nature as possible.) 2, 2, endothelia; 3, a stomata verum, one of the cells of which shows a nucleus.

of the lymph vessel or capillary wall, as that serves as a base to investigate how fluids pass from the blood vascular system to the lymph vascular system. We may discard the idea that the fluid passes to any extent through the endothelial plates of either blood or lymph vessel.

Hence, the route left for the passage of fluids from the blood into the lymph vessels of the peritoneum must be considered to take place by way of the interendothelial space. Whether preformed openings exist in the interendothelial space of the lymph or blood vascular system is a mooted question. There is no doubt that the blood corpuscles will wander out of blood vessels through the interendothelial space, as has been observed by many investigators. Therefore it makes no ultimate difference whether preformed openings or openings arising as required exist, so long as the fluid and corpuscles find their way out of the vessel. As far as regards the existence of preformed openings in the interendothelia of the lymph vessels, I believe they do exist.

From my experiments and investigations I am led to believe that there are both preformed openings (*stomata vera*) and interendothelial openings which arise as necessity requires. It appears to me that much more physiology lies in the interendothelial space than has hitherto been claimed. If a very high power is used on the interendothelial lines, it is plain that two dark lines exist between endothelia with a still broader intervening white line. Also that there are peculiar interendothelial bulgings, figures which seem to be constructed so that the adjacent endothelial edges when joined complement each other. The vascular interendothelial substance, and especially the lymph vascular interendothelial substance, is abundant. What is seen of the interendothelial substance after the application of  $\text{Ag. NO}_3$  is merely the finest upper edge of a dark line. The substance widens and thickens as it descends beneath the endothelial surface. Under very high power the interendothelial space may resemble a series of irregular appearing rings, with the thickened portion of the rings descending deep beneath the endothelial surface. The interendothelial space is especially large in the lymph vascular system. It stains vigorously and sharply with  $\text{Ag. NO}_3$ , and seldom in a straight line, as it may frequently do among the endothelia of the free peritoneal serosa. Zigzag, sinuous lines like the cranial suture outlines characterize the silver-stained interendothelial space of the lymph vascular system. They are so distinct that they may be frequently very plainly observed through the transparent endothelia of the free peritoneal serosa over the *zona tendinea* of the diaphragm.

The form and arrangement of the endothelia of the lymph vascular system and the form of the endothelium constituting the wall of the lymph vessel are varied in different animals. The arrangement varies considerably even in the same species of animals. But in general, one can say that in analogous localities of the peritoneum analogous forms of endothelia repeat themselves. The form and arrangement of the endothelia of the lymph vascular system may be well compared to the

endothelia of the blood vascular system, as the lymph system peculiar to vertebrates is a derivation of the blood system. What is said here will serve for much that might be said on the same subject in regard to the endothelia of the blood vascular system.

1. The wall of the blood vascular capillaries and the wall of the lymph vascular capillaries may be essentially the same, viz.: composed

of a single layer of endothelium so placed edge to edge that closed or periodically closed tubes or irregular spaces are formed, the walls of both vascular systems being independent and isolable from the adjacent tissue. It is freely admitted that the lymph capillaries are much more difficult to isolate than the blood capillaries from the surrounding tissue.



FIG. 165.—(Author.) Young dog's gastro-splenic omentum (oc. 4, ob. 3, R.). It illustrates the lymphatic vessels. It shows lymph capillaries or sinuses and connective tissue corpuseles. 1, 1, 1, stomata vera; 2, 2, 2, stomata spuria; 3, 3, 3, origin of lymphatic vessels; 4, 4, 4, 4, new germinating endothelia stained dark brown with Ag. NO<sub>3</sub>. The figure is drawn very carefully after nature, except a few endothelial cells, which were nearly swept away and were repaired. 6, lymph space between vessels; v, v, valves.



FIG. 166.—Pigment cells lying immediately under the peritoneum on the lymphatica cisterna magna of the frog.

2. The large lymph vessels as well as the blood vessels are distinct tubes composed of (a) tunica intima; (b) tunica media, (c) tunica adventitia. The endothelia lining the vessel, blood or lymph, i. e., the tunica intima, are composed of similar forms and figures. The lymph and blood vascular endothelia are directly comparable. The wall of the lymph capillary is composed of flat, nucleated, irregularly bordered cells. We will consider three points in the form and arrangement of the en-



dothelia in the lymph vascular system (a) the shape; (b) the lineaments and (c) the grouping of the endothelia. One of the most characteristic matters is the grouping of endothelia. This can be noted not only in peritoneal endothelia, but in endothelia of lymphatic vessels and sinuses.

The grouping must evidently be a primordial matter, an arrangement to suit preformed structures—stomata vera. Since I have carefully examined the endothelia of the foetal pig, I am more impressed that endothelial grouping is to fit preformed openings—stomata—and that the stomatal ring is not circumscribed by the nuclei of the adjacent endothelia, as announced by a German investigator, the lamented Schweigger-Seidel (1834-1871). The grouping of endothelia must be for a purpose at least. It will lend peculiar shape to the concerned endothelial cells. It suggests function to some center, and that center is the stoma which controls fluid, allows opportunity for cell growth and nourishment. It is insufficient to say that the stomata are the mere result of contraction of interendothelial substance at the common junction of several cells.

The characteristic of the border line of lymph endothelium is sinuosity—an uneven, irregular, serpentine line resembling the cranial sutures. The border is often irregularly serrated. However, the border-line of lymph endothelia may not always be serrated. The line may be smooth, and present round, oval bulgings, large loops with small necks. The necks of these loops may be so narrow that the lines almost touch each other. No doubt that when the lines of such necks come in contact they constrict off round apertures, which may appear like stomata spuria. The cell borders are sometimes so irregular, presenting shallow and deep bulging loops, that the endothelial cell may present the shape of a star. The cells may look like the leaf or branch of plants. Then, again, the borders may be both smooth and sinuous. All kinds of grades arise. In the endothelia of the lymph vessels the sinuous borders prevail. One can find both kinds of smooth and sinuous endothelial borders forming the wall of the same lymph vessel. The smooth endothelial borders arise in the walls of wide lymph vessels and also in the widest part of narrow vessels. In the beginning of lymph capillaries endothelial borders are very sinuous, serrated and irregular. On the gall-bladder of a frog I found endothelia so looped that if the neck became much more constricted it would constrict off smooth, round endothelial cells. It appears that such constricted off round cells have been named stomata spuria, especially if they be small. The constricted off round cell has been thought to be a young endothelial cell. Some of the lymphatic endothelia are enormous in length, and Auerbach claims that the endothelial cell of the lymph vessels may

have a long diameter, ten times as long as its shortest diameter. I have found endothelia of lymphatic vessels with such disproportionate diameters or larger. Yet the long diameter often exceeds the short only by several folds. By carefully watching the microscopic field of the lymph vascular endothelia, occasionally one notices remarkable irregular endothelia as to size which, so far as I can find, is generally on the border between lymph channels and capillaries, or such may be observed

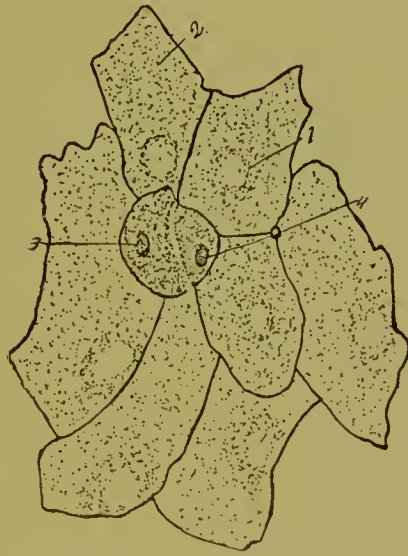


FIG. 167.—(Author.) Is a sketch on which much care was used to draw it as absolutely natural as possible. It is from the cisterna lymphatica magna of a frog, silvered with one-half per cent. solution. Observe how the shape and outline of the endothelia differ from those of the other frog, as both specimens are from the lymph sac on the right side of different frogs. 1, points to the nucleus which is not well stained in the center. 2, points to the endothelial cell browned by the silver (perhaps the pepper-and-salt appearance is the precipitated albuminous substance). 3, points to a nucleus in a granular young cell of a stoma verum, and 4 shows the other nucleus of the granular cell. A point may here be noted in this stoma verum. Notice that one of the intercellular black lines passes directly across the stoma. Now, in other specimens it may be observed that the granular cells may be entirely below the stoma verum and be gradually projecting up between the junction of the several cells, i. e., the granular polyhedral cells of the stomata vera are a matter of slow growth. Observe the nuclei are excentrically located. No stomata spuria are marked in this specimen except where the line crosses. The stoma verum is a vertical lymphatic canal which maintains a direct open communication between the peritoneal cavity and the subserous lymphatic vessels. The stomata vera are regulators of serous fluid. They are the source of young endothelia to supply decaying ones.

at the junctions of lymph vessels or where a narrow vessel becomes suddenly wider. Such irregular lymph endothelia is due to some mechanical force, e. g., excessive fluids producing expansion.

The stretching of the endothelia forming the walls of lymph vessels, due to filling and emptying of the vessels, produces remarkably shaped cells. Some have long, pointed ends which fit into the depression of other cells. One to three pointed processes may be found on a single endothelial cell. If one will move the slide containing the lymphatic

vessels in various directions, the different outlines of lymph endothelia may be observed. The best are seen on the peritoneum after staining with Ag. NO<sub>3</sub>, subsequently brushing off the peritoneal endothelia. In general three grades are observed:

1. The endothelia covering the origin of the lymph capillaries. These are very sinuous and serrated in outline, and the various diameters do not exceed each other very much. Yet the cells are very irregular in size.

2. On passing from the edge of the peritoneal endothelia to the lymphatic endothelia covering the lymph vessels, the cells begin to elongate and the borders are not so sinuous or serrated. The long diameter of the endothelia begin to exceed the short diameter double or more. The sinuous or serrated interendothelial lines show themselves more like loops, round bulgings of the cells. In short, the lymph endothelia begin to increase in size perhaps from the changing calibre of the vessel. The endothelia of the lymph vessel being very thin, the lymph vessel is subject to small contraction and wide dilation.

3. Finally, one can observe in the middle of long lymph vessels and in dilated localities of lymph capillaries very long, narrow endothelial cells. The endothelia are very irregular in shape. Their form is that of deep bulgings of their border, or rather loops may be so prominent and their necks so narrow that but little further constriction of the neck would produce an additional endothelial cell. Perhaps many are formed by the neck of a loop being constricted off. In this largest grade of cells the long diameter exceeds the shortest by several fold. Some of these enormous giant lymphatic endothelia may be easily observed in the frog's peritoneum, in the diaphragm of the rabbit beneath the serosa of the diaphragm. If one secures the tendinous zone of the human diaphragm soon after death, and is fortunate enough with Ag. NO<sub>3</sub> and light to produce a typical specimen, he may be able to observe under a high power all the wide variations of the shape and size of the lymph endothelia through the transparent peritoneal serosa. Many of the wide lymph channels in the human diaphragm will show very uniform elongated spindle-shaped endothelia.

The peritoneal endothelia are in general much more regular and uniform than the endothelia covering lymphatic vessels. The only reasons I can offer for this great irregular shape of endothelia covering lymph vessels, and I have seen no explanations, are (a) that the endothelia are very elastic and the elastic fibres are in a soft, plastic state which could by degrees adapt itself to almost any shape and size, and (b) the lymphatic vessels have a widely variable calibre owing to varying contents. The significance of the various sizes of the endothelia of lymph vessels and spaces is unknown. At first, as one studies superficially the endo-



thelia of the lymph vessels, the impression arises of chaotic irregularity. But by systematic study of large numbers of different sections of the lymph vessels in different species of animals, one will finally be impressed that the endothelia of lymph vessels are analogous in analogous localities of the subperitoneal region. It is true that there are localities in lymphatic vessels where the endothelia are suddenly distinguished by a wide difference from the adjacent ones, but this is generally where the calibre of the lymph vessel is suddenly dilated, artificially or temporarily, it may be pathologically. It may be stated that the internal walls of the

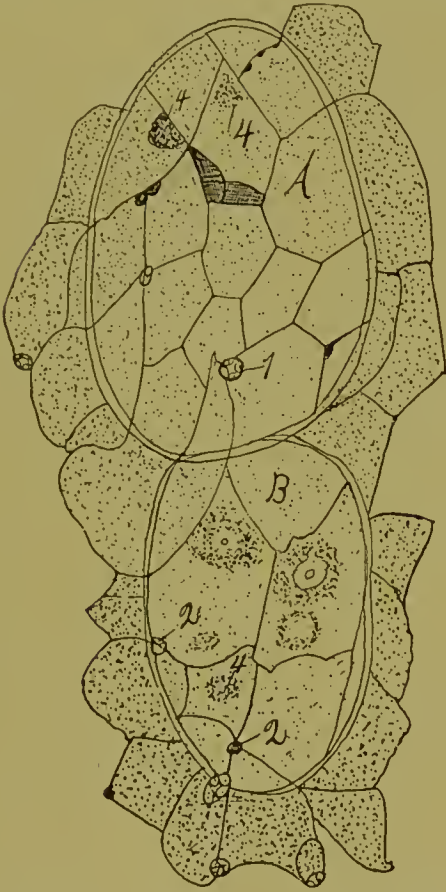


FIG. 168.—(Author.) Gastro-hepatic omentum of a woman of 45. Two vacuolated cells which are widely expanded, almost sufficiently to be called a lymph sinus. A has five stomata vera. B has two stomata vera, 2, 2; 3, another vacuolated cell begins. (Oe. 2, ob. 8 a, R.) Ag. No. 3 applied. One of the best places to study vacuolation is on the adult human omentum, especially along the large trabeculae; 4, 4, 4 are very brown spots. In these germinal tracts the endothelia are of all sizes and shapes. This was taken from a region where numerous vacuolated cells existed of all sizes.

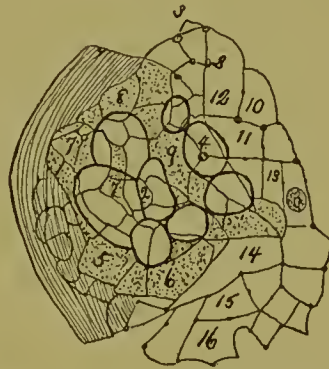


FIG. 169.—(Author.) Woman's omentum majus. Eight vacuolated cells. (Oe. 4, ob. 3.) 1, An endothelial cell on the bottom of the lymph sinus or capillary very much browned, as well as 2 at the bottom of another stomata verum in lymph sinus endothelia; 4, stomata at bottom of large sinus or vacuolated cell; 5, 6, 7, 8, 9, germinating endothelial cells around the circumference of the lymph sinus or vacuolated cells; 9 also shows the brown growing endothelia between and among the vacuolated cells. 7 is doubtless a karyokinetic figure. Note that the growing brown lymph endothelia of the vacuolated cells is continuous directly with the lymph endothelia (10, 11, 12, 13, 14, 15, 16) to the right. i. e., these seven vacuolated cells will soon form a new lymph sinus or capillaries. The vacuolated cells are widening stomata.

lymph vessels are lined by endothelia, and these endothelia are definitely marked off by Ag. NO<sub>3</sub> staining.

It is not entirely clear why the endothelia of the lymph vascular system assume such elongated spindle-shapes while the endothelia of the free peritoneal serosa are so nearly polygonal. Yet similar spin-

dle-shaped endothelia are found in the blood vascular system, due doubtless to contraction and expansion of the vessels. Also that the shape, outline and grouping of the endothelia of the lymph vascular system are analogous in corresponding places in the same species of animal.

The thinnest, and in fact the majority, of subserous lymphatic vessels have a wall only of endothelia, so that the staining of the Ag. NO<sub>3</sub> is just as effective whether it be applied on the external or internal surface of the lymphatic vessels, because the whole wall consists of a single layer of endothelia applied edge to edge in such a manner as to form a tube. The Ag. NO<sub>3</sub> precipitates the interendothelial substance, producing a regular, dark line. Why do the dark lines arise between the endothelial cells forming the lymph vessels? "It is due," said the memorable Von Recklinghausen, "to a precipitation of albuminate of silver which lies between the adjacent endothelia." In a generation we have not changed Recklinghausen's opinion. Some contended that the dark interendothelial lines arising on the application of Ag. NO<sub>3</sub> are elastic fibres, others that they are furrows in the serous surface. The dark lines may be so fine that a high power is required to see them, or the interendothelial lines may be so broad that they will reach almost to the nucleus of the endothelial cell. I have observed this very condition for weeks in certain specimens. If one stains the endothelial membrane very lightly, it can again be stained later heavily.

The breadth of the interendothelial lines of silver albuminate depends on (a) the concentration of the Ag. NO<sub>3</sub> and (b) on the duration and condition of light. To stain endothelia is a species of photography, and we may expect the light to act similar to such processes. The stronger the silver nitrate the broader the lines. I have watched the specimens brown day by day until almost the whole endothelial surface was of a deep, dark brown, the center of the nucleus being of the lightest shade. It may be noticed on some endothelial specimens that at first the fine double lines are even, but after a few days the intercellular lines present a broken, dotted appearance. Perhaps the intercellular space had contracted, leaving places unfilled by albuminate of silver.

It is no longer a matter of doubt that the dark, interendothelial lines produced by Ag. NO<sub>3</sub> are the borders of the endothelia of the lymph vascular system. By careful staining and a very high power one can observe that the single dark interendothelial line will become very plainly two dark lines, with an intervening broader white space. Each single dark line, albuminate of silver clings closely to the edge of its respective endothelial plate. Peculiar bulging structures exist in the interendothelial substance which, when the two endothelial plates join edge to edge, complement each other. Under a high power these inter-

endothelial structures look like a row of rings whose subperitoneal portion is larger and thicker than the portion more on a plane with the endothelia of the free peritoneal serosa. This appearance is no doubt due to the fact that the precipitable interendothelial albumen increases as it descends into the peritoneal bed, and also that the more peritoneal the interendothelial substance is, the fresher or younger it is, and hence the more sensitive to the silver salt. As the days of sunlight spread their rays on specimens the lines broaden, and often the interendothelial lines become irregularly interrupted, broken and acquire sharper angles and projections. This is doubtless due to the silver salt



FIG. 170.—(Author.) Young dog's lymph sinus, or well-developed vacuolated cell in a region of germinal endothelial cells. 1, 1, 1, open stomata on the lymph sinus; 2, 2, 2, endothelia covering the lymph sinus or vacuolated cells; 4, 4, 4, show the edge of the vacuolated cell or lymph sinus is brown and hence young protoplasm. Note the very brown endothelia surrounding the vacuolated cells (4, vacuolated cells, Ag.  $\text{NO}_3$ ). The vacuolated cells continue to multiply and form lymph sinuses. Observe how the endothelia enlarge as they diverge from the lymph sinus border.



FIG. 171.—(Author.) Omentum of woman aged 30, twenty-four hours after death. Ag.  $\text{NO}_3$ . (Oc. 4, ob. 3, R.) This is an interesting specimen, showing two vacuolated cells. 1, 2, 3, 4, 5, show the common endothelia brushed off, and lymph endothelia irregular, and many stomata spuria; 6, 7, 8, 9, 10, 11, stomata vera of lymph capillaries. 1, 2, are become lymph sinuses or capillaries. The endothelia adjacent to the vacuolated cells 1 and 2 (or lymph sinuses or lymph capillaries) are chiefly of a germinal character, but some resemble common endothelia in character.

gradually precipitating the older and less sensitive albumen, which requires a longer line. It should be expected that the silver salt only attacks the most exposed surface of the interendothelial substance and by gradually attacking the lower strata precipitates it.

Right here comes a matter I observed in the use of the silver salts on the interendothelial space of the lymph vascular system. It produces more rapidly, and a broader dark line in the interendothelial substance of the lymph vascular system than in the interendothelia of the free peritoneal serosa. Doubtless this is due to the silver salt being capable of attacking more surface of the interendothelial space of the



lymph vessels than it could of the interendothelial space of the free peritoneal serosa. Besides, it appears that the interendothelial space of the lymph vascular system is younger and more sensitive fluid bathing it than that of the interendothelial space of the free peritoneal serosa.

The shape and outline, form and lineaments of the endothelia of the lymph vascular system of the peritoncum do not appear to be significant except that the more interendothelial substance presented the more physiologic function it is capable of accomplishing. However, the grouping of the endothelia of the lymph vascular system is of a far more significant nature, as indicating primordial conditions, preformed structures, with certain functions, as structure and function in the animal economy are inseparable. It appears no longer tenable to hold the dark, irregular interendothelial lines of the lymph vascular system precipitated by  $\text{Ag. NO}_3$  to be either elastic fibres or precipitates in furrows on the endothelial surface, nor can one deny existence of the interendothelial substance entirely, as was early done by Robinski and later authors. It may be gracefully admitted that the signification of the action of  $\text{Ag. NO}_3$  on the interendothelial space is not fully settled.

In the interendothelial space of the lymph vascular system one can observe two kinds of openings, viz.: (a) stomata vera and (b) stomata spuria. It is evident that the lymph vessels must necessarily possess some form of openings in order to allow the lymph fluid to pass into them from the blood vessels, i. e., if we exclude the endothelial plates of the lymph vessel as permitting the fluid passing through their walls. The exact nature and location of these openings have concerned many observers, as Recklinghausen, 1862; Teichmann, 1862; Oedmansson, 1863; Auerbach, 1866; Ludwig and his pupils, 1866; Dogiel, Schweigger-Seidel, Dybkowsky, Chrzonszewsky and his pupils, especially Brucke, His, Frey and Affannasiew, 1866; Lawdowsky, 1870; and Klein and Burdon-Sanderson, 1872. Also should be mentioned the work of the Italians, Bizzozero, 1873; Salvioli, Maffuei, Muscatello and others. Among the French, Ranvier, Tournaux, Hermann, Dubar, Remy, and Robin; the Austrian, Prof. Beck, and the Russian, Prof. Kolossow, 1894, having produced excellent articles published in German. The above names constitute the landmarks in the progress in the discovery of the lymph vessels of the peritoneum during the past generation, a galaxy of names unsurpassed.

The openings in the lymph vessels are designated stomata vera or spuria. Arnold called the large ones stomata and the small ones stigmata. We will say that a stoma verum is located at the common junction of three or more lymph endothelia. It is true it may not be a

stoma verum, but merely an aperture. But all stomata vera are at the junction of several endothelia. A stoma spurium is located on a single interendothelial line but, doubtless, many are stomata spuria which exist at the common junction of several endothelial cells. With this limited and incomplete definition, we will discuss the openings in the lymph vessels as seen from the peritoneal serosa.

The stomata vera are lined at their upper end by granular, polyhedral, nucleated cells, which stain quite dark with  $\text{Ag. NO}_3$ . The number of granular cells which lines the mouth may be made out to be two



FIG. 172.--(Author.) From rabbit's omentum majus. A typical lymphatic sinus surrounded by typical germinal endothelia. 1, 1, 1, the lymph or capillary sinus; 2, 2, 2, a secondary lymph sinus; 3, 4, 5, 6, 7, the germinal endothelia (note the three elongated cells at 8); 9, closed stoma verum. It appears that such lymph sinuses arise by the vacuolation of cells; the cells by repeated vacuolation form large, numerous, and irregular endothelial plates; here they have really become a lymph channel. (Oc. 4, ob. 3. R.) This drawing is taken from a vast region of germination. Note how the endothelia enlarge as they recede from the sinus, 1, 1, 1.



FIG. 173. (A)—(Author.) Drawn from the peritoneum of a wood-pecker under high power. It represents a lymph vessel with one stoma vera. The nucleated cells forming the lymph vessel wall are plain. 1, stoma verum surrounded by eight nucleated polyhedral granular cells; 2, 3, nuclei of the endothelia of the lymph vessel wall. The interendothelial space consisting of two parallel lines and transverse anastomosing processes is very wide in birds. 6, 7, 8, stomata vera closed.

FIG. 173. (B)—Drawn from a turtle's mesogaster under high power. 9, stoma verum surrounded by 11 granular, nucleated cells; 11, endothelia; 10, 12, nuclei.

or more in number. The number 4 is frequently observed, or 5 or 7. However, the stomata vera of the lymph vessels do not in general possess as many granular cells lining the circumference of the stomata as those found in the peritoneal serosa. The number of granular cells lining the mouth of these canals is very variable in any locality. Some have asserted that the small cells or plates surrounding the stomata are endothelia which have remained congenitally small, i. e., never grow to adult size. This is improbable, for if one looks at many stomata the granular cells are all about the same size, no varying grades of size

present. One of the matters which suggests doubt as to the nature of these granular stomatal cells is that no nucleus could be discovered in many of them, yet in some I have noted several spots which I considered nuclei. Now, if these stomatal cells are not cells, i. e., possess no nuclei, what are they and what is their source? Auerbach's explanation that these small, stomatal, granular cells arise by loops of the endothelial plate becoming constricted off is, in my mind, untenable, formerly constricted off loops of the plate would not change their behavior with reagents. These stomatal plates are very granular, cuboidal in shape or polyhedral, and stain highly dark red by the application of Ag. NO<sub>3</sub>. Now, in the examination of foetal and young lymph endothelia, though they do not entirely resemble adult endothelia, yet they are entirely analogous in corresponding places of adult and foetal life. Birdsall, in Satterwaite's histology, makes the erroneous statement that the stomatal openings do not exist before birth. I have found them in the embryotic pig and in the human child at birth. It is also in accord with investigations to say that the stomatal cells are merely the nuclei of the surrounding endothelial plates. Auerbach makes another assertion which is very unlikely, and that is that there exists a non-uniform plastic condition in the endothelial plate, that the non-uniform growth forces small loops or peninsulas of the endothelial plate into the space of the common junction of several cells, and then the projecting piece of plate becomes broken off. Hence, all the granular cells of the stomata are broken remnants of adjacent cells, a view which I think will not stand the test of investigation. How would broken pieces of cell live, reproduce and grow? Such haphazard formations could never produce definite structures like stomata vera. Of course, it is easy to assert that a stoma spurium looks simply like a widened place in the interendothelial line, and still more so when a bright spot arises surrounded by a black ring. If the stomata spuria are not points where connective tissue corpuscles, white blood corpuscles, reach the surface, then some other function must be attributed to them. It must be stated that the irregular relation of form, size and distribution of stomata speaks in general against distinct anatomical structures. Some stomata are round, others oval, and others spindle-shaped, and still others half-moon shaped, elongated and even sinuous, but likely much of the irregularity of shape is due to trauma or dragging. Large fields exist without a trace and then a field arises with numerous stomata. It appears to me that many endothelia of lymph vessels have openings in their walls. But the chief argument against the opening in the lymph vessel walls as being stomata is their irregular occurrence and absence in places where they should occur. It may be that I am like many other investigators who try to fit cause and effect to precon-



ceived notions. For example, it is natural to think that our views of physiology demand the existence of openings in the blood and lymph vessel walls so that fluids can pass from one into the other. Hence, in seeing certain structures or openings in the walls of those vessels we are likely too apt to attempt to force such into our lines of cause and effect.

In regard to the walls of the lymph channels we may state: 1. The larger vessels consist of a lining membrane composed of endothelial plates arranged edge to edge in such a manner as to form a closed sheath. Besides, the larger lymph vessels have a tunica intima, a tunica media and a tunica adventitia exactly as veins and arteries.

2. The so-called capillaries of the lymph vascular system consist essentially of a single layer of endothelia arranged edge to edge so that a closed tube is constructed. It appears to me from the silver staining that many lymph capillaries, vessels, tubes or lacunae, as in the frog and turtle, have no other wall than a single layer of endothelial plates.

3. Beyond these two kinds of lymph vessels, the large vessels and



FIG. 174.—(H. B. for P. L. 1873.) Section of mesenteric gland of ox which has been hardened in Muller's fluid and then shaken. a. Capillary nucleated cells representing the nodes of the delicate reticulum adenoid tissue.

the capillaries, there exist others which I am so far unable to make positive assertion. These vessels or canals mediate between the blood vascular capillaries and the lymph vascular capillaries. They have been called sap canals, juice canals, lymph canaliculi, serous canals and interstitial canals. But after observing many kinds of specimens of the peritoneum extending over many months, I am still unable positively to assert whether I can distinguish endothelial walls to these serous canals in all the specimens. One can distinctly and directly observe a connection between the lymph canalicular system and serous canals and the lymphatic capillaries, demonstrating to the eye that fluid can pass from the serous canals to the lymph capillaries without obstruction.

Experimentation first discovered that  $\text{Ag. NO}_3$  (Reeklinghausen) would stain the interendothelia of lymph vessels. Experimentation first showed that lymph vessels could be distended and traced, and experimentation finally taught us the function of the peritoneum. The addition of the enormous lymph system to the peritoneum makes it in

reality a great lymph sac. Hence, all function of the peritoneum must have more or less reference to the bed of lymph vessels on which it rests. The subjects I chose for demonstrating the function and physiology of the peritoneum were rabbits, frogs, turtles and dogs and the best material was Berlin blue suspended in an alcoholic watery solution to which is added a physiologic salt solution having a body temperature. This solution was injected slowly into the peritoneal cavity and allowed to remain for 10 minutes to 26 hours before the animal was killed. This is a natural physiologic method disassociated from diseased process, and capable of indicating the real action and function of the peritoneum. I used various quantities of the colored fluid at blood heat for the injection.

The injection does not appear to produce much shock. But evidently the animal is quite ill in the succeeding 24 hours, with apparent increase of illness on the 2nd day. The keeper, who often saw the rabbits used after the injection, considered that they were suffering and did not keep them after the 2nd day, so no report of results is to be had beyond that time. On killing the rabbits after the injection, the effect on the peritoneum is found to be according to the length of time the injected material is allowed to remain in the peritoneal cavity. As I was not investigating pathologic effects, but solely physiologic function, the rabbits were killed as soon as physiologic function could be well detected. One can well observe physiologic effects in 10 minutes, and still more definite in 6 to 8 hours. Injections remaining longer than 15 hours in the peritoneal cavity show distinct abnormal (pathologic) conditions, as enlargement or swelling of the corpuscles, increase of fluid separating elements, fluid distending the vessels, slight cloudiness of the surface endothelia and a cloudy swelling of the interendothelial space. Also the endothelia begin to swell and become gradually detached from their bed. The bed leaves a depression in the center, while the circumference of the endothelial cell is elevated and has a definite rim of darker matter which looks as if the upper part of the endothelial plate, i. e., the cover-plate, was forcibly torn from its bed. The hardened portion of the endothelial plate floats off as a thin reticulated membrane, leaving a pit behind. Now, even six hours after the injection of the coloring fluid into the abdominal cavity, changes can be detected with the naked eye (congestion) and very easily with the microscope. What becomes of the colored injection into the peritoneal cavity?

In the first place, the great omentum has a wonderful tendency to roll itself around the invading mass. It attempts to corral the mass of matter and circumscribe it. It is a wonderful peritoneal protector, moving to invaded parts to check foreign progress. The omentum rolls

the mass into strings, cords, balls, and then wraps itself around it. It is evident that the omentum majus is a peritoneal protector, a guard against invasion, a man-of-war to defend ports of access. The unfortunate trouble with the omentum is that when it has defended one severe inflammation, it becomes ever afterward crippled by fixation and dislocation. It has laid down its chief weapon of defense, which is motion. It has become disarmed, and like a crippled, pensioned soldier, it is confined to a small office, a remnant of a past war of inflammation.

The second observation after the peritoneal injection of coloring matter is the appearance of swarms of leucocytes. This appearance of leucocytes is the chief phenomenon in the whole experimentation. The leucocytes swarm out on the surface of the peritoneum shortly after the coloring fluid is injected into the peritoneal cavity. It is a remarkable occurrence. The meaning of the leucocytic hosts is that it is the method of peritoneal defense against invasion. The leucocytes englobe, surround, destroy or digest the invading host, and thus become defenders of the peritoneum. All animals possess visible weapons of defense for bodily safety against other animals. Teeth, horns, claws, hoofs,

FIG. 175.—(F. Schweigger-Seidel and J. Dogiel, 1866.) Represents the peritoneal surface of the lymph sac of a frog. The author designated this cut by the title "*Bauchen hoehlenflache der scheidenwand mit gruppen von kleineren flummerzellen.*" They called the groups of small cells ciliated cells, but they are no doubt what we now designate after E. Klein as germinal endothelia. One can observe vast areas of such small cell groups of 1 and 2 on frog's peritoneum.



wings and legs are weapons easily seen and comprehended as valuable means to survive in the conflict and struggles of life. Some animals can be gradually injured, immuned to meet forces by slowly changing environments or by inoculation, but in each case it is a method of defense for survival. Now, among all animals there is an invading foe to life, a force against survival. This enemy of life is liable to possess a defender. The enemy of life in this case is inflammation (infection, foreign bodies) and the defender the leucocyte. The invasion is infection and the defense is the leucocyte. Metchnikoff has shown as far as he could discover that all animal organism has to fight invading parasites. From unicellular animals up to complicated man there is a continuous process menacing life, known as inflammation (infection). It has induced the animal organism to produce methods of defense. As this inflammation or parasitism is a continuous process through all animal life, the method of defense—leucocytosis—is a purely evolution-



ary process, which has advanced step by step with animal changes and complications, environments playing a prominent role, new forces move against the survivor. It is not strange that animal life should develop methods of defense against its chief foe—*inflammation, infection or microbe invasion*. Animal and plant life are the chief enemies of each other. Now, in the injection of liquid coloring matter into the peritoneal cavity, we have a typical scene—a battle of leucocytes against the invading particles of Berlin blue. The leucocytes swarm out of the peritoneal walls and attack the particles of blue by surrounding them, englobing them, and finally transporting them through the diaphragmatic serosa into the vast bed of lymph channels situated in the wall of the diaphragm. One can notice the particles of Berlin blue scattered along the diaphragmatic lymph channels either free or englobed within leucocytes. The leucocyte passes out of the lymph vessels or blood vessels and wanders about until it comes in contact with a particle or particles of blue, and then it wanders back into the lymph vessels of the diaphragm. If the leucocyte is not big enough to surround the blue particle, one or several more come to the aid and they gradually surround or englobe it. By some motor means they then transport their large prize through the serosa into lymph channels in the diaphragm. So far I can only find these leucocytes containing Berlin blue in the lymph channels of the diaphragm. I have not found them in any other peritoneal lymph channels. The explanation that Bizzozero gives of the lymphatics of the diaphragm being the only depository for the Berlin blue, is that the *membrana limitans* covering the *pars tendinea* of the diaphragm possesses many apertures through which the blue could be carried by the leucocytes. The *membrana limitans* in portions of the peritoneum, as far as examined outside of the diaphragm, possesses no apertures. Whatever be the explanation, the particles of Berlin blue are transported by the leucocytes only into the lymphatics of the diaphragm. Hence, there must be a current or motion toward the diaphragm. Sometimes there will be found on the diaphragmatic serosa large masses of leucocytes which are thoroughly filled, peppered as it were, with small particles of blue but have not passed into the diaphragmatic lymph channels. Again, one can find large masses of leucocytes thoroughly filling with particles of Berlin blue lying free in the diaphragmatic lymph channels. In such cases hosts of leucocytes have coalesced in fighting the battle and imprisoned the hosts in safe localities.

How and why do these leucocytes swarm to the peritoneal cavity? It may be stated that any irritation to the peritoneum, mechanical or infectious, induces the leucocytes to collect where such mechanical or infectious irritation exists. It may be said that inflammation is always

accompanied by leucocytes just as sure as the shadow follows the substance. It is the nature of the leucocytes then to assemble at points of irritation. They are volunteers enlisted for defense. They are the methods by which organized tissue defends itself against invasion. The leucocytes are tissue protectors. They are developed by an evolutionary process for the distinct purpose of combating inflammation, the chief foe of animal organism. The leucocytes are the body-guard always on hand, always under strict discipline for immediate march to the attacked frontier. They are like a man-of-war, ready at a moment's notice to sail to invaded parts to protect unfortified tissues. Leucocytes are as necessary as food. They are nature's standing army to insure self-preservation. Irritation in the peritoneum is like pressing a bell-button, it rings up the whole system, ordering any army of leucocytes to march to a definite point. In the rabbit it is easy to note the vast hosts of leucocytes which the Berlin blue calls into the field of irritation of the peritoneum. They swarm around the foreign particles, and when one leucocyte is unable to englobe a particle others rapidly

FIG. 176.—(Schweigger-Seidel and Dogiel, 1866.) Illustrates a portion of the peritoneal surface of the cisterna lymphatica magna of a frog. 1, endothelial plate; at 2 may be seen a stoma verum surrounded by small protoplasmic polyhedral cells. The theory of Schweigger-Seidel was that each one of these small protoplasmic cells was the nucleus of the endothelial plate, i. e., the nuclei of the adjacent endothelial plates, which have a common border around the stoma, have shifted to the edge which borders the stoma. There are two other stomata vera. Many times have I noted specimens which contradict Schweigger-Seidel's theory.



come to the rescue. Yet sometimes many particles will be englobed in one leucocyte. Leucocytes have such a tendency to unite, to coalesce, that it is difficult to say how many are included in a mass of protoplasm infiltrated with small particles of the coloring matter. Hence, the leucocytes assemble at points of irritation because their business is defense. It is their nature to be protectors of tissue, trained through thousands of ages. They are the instruments to combat inflammation, the means of animal life for self-protection and for existence. Leucocytes are nature's only methods of continued self-preservation to the organism and to put the animal organism on guard. To say that leucocytes have the power to migrate to a point of inflammation is endowing them with no more intelligence than to say that red blood corpuscles have the intelligence to carry  $\text{CO}_2$  toward the lungs and  $\text{O}_2$  away from them. It is simply announcing their function. It is the function of

the leucocyte to combat inflammation and that of the red blood corpuscle to carry  $\text{CO}_2$  or O. Nerve cells and nerves have the intelligence or function to receive sensation and transmit motion which is a complicated process, but they are endowed with such power, intelligence and function. Now the function, power or intelligence of the leucocytes is to combat inflammation by (a) migrating to the seat of invasion or inflammation. It does not matter whether it be infectious or non-infectious, i. e., septic or mechanical. The leucocyte does not seem able to discriminate, but he is on hand for an emergency. (b) The leucocyte surrounds or englobes the foreign body, it matters not what it is. It matters not how many leucocytes it requires, they attempt to entirely surround it and make it enclosed or englobed away from tissue which otherwise it would irritate. (c) The leucocyte attempts to digest the invader, and for this purpose surrounds it with its protoplasm. At any rate, the invader of the peritoneum becomes a prisoner in solid walls of protoplasm. Pathogenic or non-pathogenic, he is a disarmed prisoner of war to be disposed of in the future. If the process is not digestion, it is a process of destruction by secretions of the leucocyte. The leucocyte wanders out of the lymph space and blood vessel to meet invaders, which it attacks by burying the invader, digesting him, imprisoning or sterilizing him so that reproduction or multiplication is impossible.



## CHAPTER VII.

### THE NERVES OF THE PERITONEUM.

"A man will not be observed in doing that which he can do best."—*Emerson*.

Haller described the nerves of the peritoneum in 1751 and Malpighi confirmed his labors. Bajard, in 1818, said the nerves of the peritoneum could not be followed out. Later, Luschka and Bourguery contradicted this opinion and maintained that the nerve filaments of the peritoneum accompany the arteries of the mesentery, the ligaments of the spleen and liver.

In 1866 Reichert said the peritoneum does not possess nerves which properly belong to it. Inzani, 1872, wrote a well recommended work on the terminations of nerves. Louis Jullien, of France, in 1873 wrote an excellent article on the termination of the peritoneal nerves.

He used :

(Auric chloride)  $\text{Au.Cl}_3$ , 0.50.

(Water)  $\text{H}_2 \text{O}$ , 100.

(Acetic Acid)  $\text{H. C}_2 \text{H}_3 \text{O}_2$ , a few drops.

Jullien notes, as all other investigators, that the specimen should remain in the solution a variable length of time, being tested during the experiment. The specimen may remain in the solution with advantage for several days, but it may be observed that the  $\text{H. C}_2 \text{H}_3 \text{O}_2$  will make it very friable. Bright sunlight provokes a distinct color deposit.

Schweigger-Seidel instituted the following formula which is reported produces good results :

Chloride of Platinum ( $\text{Pt.Cl}_4$ ), 1.

Acid Chromic  $\text{Cr O}_2 (\text{O.H}_2)$  1.

$\text{H}_2\text{O.}$ , 400.

Ranvier and Robin have studied the action of osmic acid ( $\text{Os.O}_4$ ) and silver nitrate ( $\text{Ag. NO}_3$ ). We may see in the peritoneal specimens nerve trunks, branches and terminations of medullary fibres, non-medullary fibres and nerve endings. They accompany the vessels most often arteries and veins, and travel with the arteries and veins under the endothelia. To prove this, desquamate the endothelia with acid or brush and one will observe the nerve fibres bared and undisturbed. The nerve fibres course chiefly in bundles, but may form a mesh-work.

Henry J. Berkely (1895) in John's Hospital Reports presents some excellent labors on the staining of nerves. The drawings accompanying the article deserve the highest commendation. Berkely's elegant drawings impress us with the ideas of Pflueger who, nearly a generation past, announced that every liver cell was penetrated by a nerve fibre. The cells of the peritoneum perhaps differ from the liver cells only in being less active and require less amount of nerve supply. However, it would appear from microscopic work with effective staining material that every endothelial cell is supplied by nerve terminals. The nerves are apt to reach the cells through interstices of the tissue, and one is apt to find the nerve fibres stained so as to be visible in the interendothelial spaces. Any organs, like cells which have the power to expand, to contract, the power to reproduce and assimilate, must doubtless be supplied by the most delicate known apparatus, which is a nerve terminal. Nerves in any portion of the body tend to follow the parts which demand control, as especially the blood vessels, and the blood vessels are only a compound of cells. But it is on certain reagents that we must at present rely for the demonstration of nerves, as silver, gold and osmic acid. Pflueger worked with osmic acid in 1869 and Nesterowsky did excellent work with gold in 1875. By careful staining one can demonstrate that the small arteries of the peritoneum have spun about them fine networks of nerves. Some small arteries have such an extensive expansion of the nerve network about them that we may entertain reasonable doubts whether all the strands brought into view by the reagents are really nerves. The nerve network about the peritoneal arteries possesses coarser and finer meshes which one may compare to the coarser mesh-work of Auerbach's plexus and the finer mesh-work of the Billroth-Meissner's plexus. In my microscopical labors in the peritoneum the gold method with acetic acid demonstrated beautifully the large new trunks, but the fine vascular nerves required much more labor in detail. However, we secured very beautiful specimens of vascular nerves presenting nerve trunks of considerable size, anastomosis by contact, coarser and finer mesh-works of nerves.

The specimens which were well and carefully stained, as with the gold staining, demonstrated conclusively that the peritoneum is an organ richly supplied with nerves. The peritoneal surface is equal in area to the skin and when injured by traumatic processes or attacked by disease shows similar effects, as profound shock, significant vascular disturbances and depressions. A square foot of peritoneum being inflamed shows similar disturbances as the inflammation of a square foot of skin.

In the peritoneum the inflammation is not so apt to be circumscribed or limited as it is in the skin, and hence the more danger of sepsis.

Sepsis may pass through the peritoneum and leave it, as a bullet leaves a gun barrel, uninjured. The sepsis affects the whole system when it gains entrance. But peritonitis, which saves life, profoundly affects the whole system by irritation of its vast and rich periphery of nerves.

The nerves found in the peritoneum are:

1. Medullated nerves.
2. Non-medullated nerves.
3. Remak's nerve fibres.
4. The Vater-Pacinian corpuscles, and other forms of nerve ending and nerve bulbs.
5. Nerve cells.

We speak of the method of preparation as one of the most important parts of our theme. I spent much time with the Ag. NO<sub>3</sub> method,



FIG. 177.—(Author.) B. Drawn from the mesentery of frog. It represents a network by contact of non-medullated nucleated nerves which end in bulbs, connective tissue corpuscles and apparently on vessel walls. Acetic acid and gold chloride preparation. 1, 2, 3, nerve cells; 4, a round bulb on the course of a nerve. A. Drawn from the peritoneal ligaments of the large bowel and bladder of a frog. Non-medullated nerves a network by contact. The nerves end in bulbs, connective tissue corpuscles and in an obscure way in the tissue. Acetic acid and gold chloride preparation. 1 and 2, connective tissue cell in which a nerve ends; 3 and 4, nerve cells; 5, nerve loop; 6, nerve endings and bulb.

but with uncertain and unsatisfactory results. Considerable time was spent with the osmic acid process, some good results, especially in demonstrating the fine non-medullated nucleated nerve fibres around the small artery or blood vessel, but with some uncertainty. The method superior to all others of preparing and demonstrating the nerves of the peritoneum is by the use of gold chloride and acetic acid. I finally discarded all other methods. The beautiful clearing process of acetic acid is not likely to be forgotten, and the distinct borders and outlines that the gold chloride furnishes to the nerve fibres is likely to be cherished by the nerve searcher. However, specimens of gold chloride are somewhat uncertain and evanescent. The method which we finally adopted was that suggested by Cyon in 1868, and is compounded and used as follows:



Take acetic acid 1 part, and  $H_2O$ . 200 parts. Of this mixture take 1,000 parts and add to it 1 part of gold chloride. Place the small portion of the peritoneum to be examined in the solution (acetic acid 1 part,  $H_2O$ . 200 parts) 1,000 parts and gold chloride 1 part, for 15 to 25 minutes, after which begin gradually to remove small pieces of the specimen into a solution of acetic acid 1 part to 200 or 400 parts of water. Allow the specimens to remain in the acidulated water from a few to 72 hours, or even longer. To examine them, snip off small pieces of the peritoneum and mount in glycerine, and place in the sunlight. The specimens are valuable to study for about 15 days, showing during that time many changes. It is of the utmost value to examine specimens which have been in both the acidulated gold chlorides for quite various periods of time. Very different phases of nerve outlines may be observed by this method. Gold chloride specimens present the most brilliant and beautiful structures seen in histology.

The best portion of the peritoneum to select for examination of the nerves is first, and above all, the cisterna lymphatica magna of the frog. Any other locality may be selected, but for ease of demonstrating typical nerves in the peritoneum, no locality is equal to the wall of the frog's lymph sac. The reason that the peritoneal nerves are so easily and certainly demonstrated in the wall of the lymph sac is (a) because the lymph-sac wall is almost devoid of blood vessels, and (b) there is scarcely a trace of elastic fibre. The nerves, though known by their contour, are easily confounded with blood vessels and elastic fibres. However, the muscle cells found in the wall of the lymph sac, appearing embryonic in type, lend some slight confusion in the examination. The omentum, centrum tendineum, mesentery and ligamentum latum of the rabbit are excellent localities for investigations of the nerves of the peritoneum. But the peritoneum of the kitten is the best of mammals to study nerves, mesenteric or omental.

In the peritoneum we have three forms of nerve fibres, viz.: (a) the non-medullated, nucleated nerve fibres, i. e., the naked nucleated axis cylinder. It consists of a finely fibrillated cord on which may be observed swellings (nuclei) at short intervals. It is the primitive axis cylinder, a continuous non-interrupted, immeasurable fine fibrillated bundle. (b) There are the fibres of Remak, consisting of a large axis cylinder, i. e., a bundle of fine, original, primitive, naked axis cylinders united into one. Also this large axis cylinder is surrounded by a very fine nucleated sheath. The large nuclei on the sheath are made visible by reagents, as gold chloride, acetic acid. This sheath is known as the neuro-lemma primitive sheath, or sheath of Schwann.

(c) There exist the medullated nerves, which consist of three parts, the axis cylinder, the neuro-lemma (Schwann's sheath) and the so-called

medullary sheath (the white substance of Schwann). The medullary sheath is of an albuminous and fatty nature, and doubtless acts as an insulator for the delicate axis fibre and nucleus.

All nerve fibres are non-medullated in the embryo, so that the medullary sheath is an acquisition of development and is not a constant factor in adult life in the peritoneum.

1. The primitive nerve fibre, the naked axis cylinder, the axial fibre or axial band, the essential portion of all nerve fibres is definitely demonstrated by the reagents,  $\text{Ag. NO}_3$ , osmic acid, but especially by gold



FIG. 178.—(Handbook for Phys. Lab., Vol. II., 1873.) The nerves of the mesentery of frog treated with chloride of gold. a, Large trunk of medullated nerve fibres. b, A single medullated nerve fibre. c and d, Non-medullated nerve fibres. e, An element belonging to the membrana propria of the mesentery. f, Nucleus of the fine non-medullated nerve fibre. g, Capillary blood-vessel. (Oc. 3, obj. 8.)

salts. In this form of naked axis cylinder in the peritoneum, it is surrounded by no sheath—neither the medullary sheath nor the neuro-lemma. The axis cylinder undergoes no interruption from the point where we discover it to its termination. It is a direct and far-reaching extension of a nerve cell. In the peritoneum this form of nerve fibre is found extensively distributed along the blood vessels. It is quite frequently found along the edge of bundles of medullary nerves or on the edge of bundles of Remak's fibres in the walls of lymph sacs of frogs and in the nerve bundles coursing through the mesentery.

It must not be overlooked that the naked axis cylinder nearer to its point of origin, i. e., toward its proximal end, may possess a medullary sheath. We can observe that the medullary sheath disappears and the nucleated axis cylinder alone is left. This is not only true of the wall of the frog's lymph sac, but of other portions of the peritoneum, so far as I have been able to trace such nerves in the peritoneum outside of the lymph sac and could note the loss of the medullary sheath. In fact, it is easy in the mesentery of the kitten to demonstrate a medullary nerve in the peritoneal membrane outside of the wall of the lymph sac. In the peritoneum the naked axis cylinder possesses fusiform or spindle-shaped swellings at short intervals. This swelling is granular, and shows a nuclens or ganglionic nerve cell. The naked axis cylinder with its nucleated granular swellings is easy to demonstrate with gold chloride and acetic acid in the peritoneum covering the wall of the lymph sac in frogs, and almost equally easy in the kitten's mesentery. There it is most typically observed running near or approximately near to the edge of bundles of nerve strands composed of medullated and Remak's nerve fibres. Curiously the naked axis nerve fibres almost always appear at the outer edge of the bundles of nerves, scarcely ever in the midst of the bundles. Again, in the peritoneum covering the lymph sac the nerve fibres will branch off into the lateral tissue and end in some other obscure manner. It often disappears suddenly. This sudden disappearing may be due to an acute bending of the nerve fibres between other tissue, it may be due to a flattening out of the nerve so that it becomes transparent. The nerve may disappear suddenly and reappear as suddenly at a distant point. The disappearance may be due to non-uniformity in coloring the nerve fibre, the non-transparent portion not responding to the coloring reagent. Finally, the fine nerve strand may have been torn in the preparation, and we lose sight of its continuity.

The above factors obscure the demonstration of the definite nerve ending in the peritoneum. In other parts of the peritoneum the naked axis cylinder may be traced to the various structures, but the fibre appears to end sometimes in a long, immeasurably fine thread, like a long-drawn-out dagger. One can trace the nerve fibre to the transverse nerve cells of a blood vessel, but no bulbous end or plate comes in sight. In short, they seem to end in tissue like the tails of seminal spermatozoa.

If one be working in a part of the peritoneum possessed with a rich supply of elastic fibre, it will be found almost a necessity to swell the ground substance by allowing the portion of peritoneum to lie in acetic acid 1 part to water 300 to 500 parts for 24 to 50 hours. Doubtless the trained expert will scarcely be deceived by the contour of a



nerve fibre, but the inexperienced better bring to his aid the acetic acid, for a fibre of extreme fineness is easily covered or obscured by dense surrounding tissue. Some say by the addition of carmine the nuclei and the fibres of the nerve will be colored, yet it cannot be denied that the nuclei and connective tissue fibre will also be colored. All this will add confusion. Gold chloride prominently attacks alone the nerve, and hence makes conspicuous the nerve alone. It is true the gold salts attack the protoplasm of cells, but by this very action the nerve shimmers through with greater conspicuity in the peritoneal tissue. It may be stated here that the writer cannot consider it always practical to transfer the photograph of nerve structures found



FIG. 179.—(Stohr, 1894.) A, Auerbach's plexus of a new-born child lying between the muscular layers. B, Meissner's plexus of same child lying under the mucosa. Magnified 50 times. g, Groups of ganglion cells; b, blood-vessel shimmering through.

in the peritoneum of the amphibian lymph sac to all other portions of the peritoneum. The nerve arrangement of the peritoneum over the lymph sac is not always comparable to the nerve arrangement in other portions of the peritoneum. Yet analogous nerve arrangements are met with in the peritoneum over the lymph sacs and other portions of the peritoneum. This remark applies especially to the nerve arrangement of the naked nucleated axis cylinder, the medullary nerves, and Remak's nerve fibres. So far in my investigations I have found the bundles of medullated and Remak's nerve fibres in portions of the peritoneum analogous to the large bundles of medullated and Remak's nerve fibres found in the peritoneum over the amphibian lymph sacs.

The naked axis, nucleated nerve fibres, forms a peculiar network in

the peritoneum which must be looked on as a significant physiologic factor. This network of nerves in the peritoneum over the lymph sac is composed chiefly of nerves which were once medullated but have lost their medulla, and what one observes in the nerve mesh-work is the axial fibre with various fusiform nerve nuclei scattered here and there. But one can observe the nerves starting at certain points in the peritoneum over the lymph sac as purely medullated bundles, but before the bundles have proceeded very far the nerves begin to divide and branch off as non-medullated nucleated fibres. The nucleus enables us to keep track of the nerve and follow it in its deviating course. However, if we turn on a very high microscopic power the naked axis cylinder, or better fibre, appears fibrillated in structure, as the bundles of medullated nerve strands wind and twist about each other as the strands of a rope. So the fibres of the naked axis or non-medullated axis wind and twist about each other. In saying that a nerve divides we mean, of course, that some of the many fibres of which the original bundle was composed simply deviate in another direction, for a primitive nerve fibre is an extension of a nerve cell uninterrupted from beginning to end.

It may be asked, What is the meaning of the nucleus situated on the axis fibre? Is it the nucleus on the primitive sheath of Schwann? In the medullated part of the same nerve, i. e., before the nerve lost its medullary sheath, we can not observe the nucleus. It is only where the nerve has lost its medullary sheath that the nucleus becomes visible. It may require more investigation in the nerves of the peritoneum to decide whether the swelling on the naked axial fibre is a nerve cell or a nucleus on an invisible sheath of Schwann. It appears to me to be a nerve cell or a nerve ganglion.

The subject of anastomosis of the peritoneal nerves may be here disposed of. The veins, arteries and lymphatics anastomose, i. e., they blend or intercommunicate with each other. For example, two or more branches so combine that the blood or lymph is finally conducted along one channel. Such a method of communication or anastomosis I think must be denied in the peritoneal nerves. No nerve strand unites with another nerve strand. If one nerve strand or fibre blends with another it simply comes in contact with it, but the nerve strand continually preserves its own identity. One nerve fibre does not anastomose with another, it simply runs alongside of it. Yet the nerves of the peritoneum over the amphibian lymph sac and in other portions of the peritoneum form a kind of network. The mesh-work of nerves is produced by the nerve fibres coursing alongside of each other, yet preserving their exact identity.

So far as regards the medullary bundles of nerves and Remak's fibres



found in the peritoneum covering the amphibian lymph sac, we can transfer the condition to the other portions of the peritoneum as typically analogous. In the mesentery of the kitten treatment with acetic acid and gold chloride I could trace the typical bundles of medullary nerves over vast areas of the peritoneum. The bundles contained 2 to 12, or more, strands which continued uninterrupted from the point of discovery to the point of sudden disappearance. The non-medullated nucleated fibre could be seen coursing along the lateral edges of the medullary bundles exactly as it could be observed in the peritoneum



FIG. 180.—(Handbook for Phys. Lab., Vol. II., 1873.) Auerbach's plexus of small intestine of human foetus, colored with gold. The plexus consisted of fibrillated substance, and is made up of trabeculae of various thicknesses, which unite in large placoids. Nucleus-like elements (unformed ganglion cells) and ganglion cells are embedded in the plexus, the whole of which is enclosed in a nucleated sheath. (Oe. 2, obj. 7.) This plexus lies between the circular and longitudinal nucleus of the gut.

over the amphibian lymph sacs. The nerve strands could be traced under the endothelial plates, but they can be seen much better with the endothelia brushed off. As the carnivora (cat) tribe possess a rich supply of elastic fibres, as well as a rich supply of connective tissue and blood vessels, it is more difficult to trace the nerve bundles than it is in the peritoneum over the amphibian lymph sac, as that is almost devoid of elastic fibres and blood vessels. The medullated nerve bundles assume a very sinuous and wavy course in the peritoneum while the single nerve strand is apt to assume a straight course. The nerve bundles,



like the uterine artery, seem to be too long for their space, but no doubt the apparent spiral excess in length is to adapt themselves to sudden motion, or stretching of the peritoneum, for the delicate nerve strands endure trauma badly.

2. The medullated nerves of the peritoneum are numerous and easy of demonstration with acetic acid and gold chloride. They are the dark bordered, double contoured nerve tubes. One of the best methods of preparation is to take a kitten of six weeks old, and place the various portions of the peritoneum, mesentery, centrum tendineum and omentum in the gold chloride 1 part, acetic acid 5 parts and  $H_2O$ , 994 parts for 15 to 25 minutes and then gradually remove the small pieces of the peritoneum into a solution of  $H_2O$ . 400 parts and acetic acid 1 part, in which preserve for a few days. To examine, simply mount in glycerine (a very slight previous silvering aids in examining the specimen).

Under the microscope the medullated nerve of the peritoneum presents three parts for examination, viz.: (a) axis cylinder, (b) medullary sheath, (c) neuro-lemma. The axis cylinder of the medullary nerve is precisely the same as the naked axis cylinder which we have just described.

The medullary sheath or white substance of Schwann is a soft, gelatinous, albuminous fatty substance which envelopes the axial fibre as an insulator. As soon as the medullary sheath is exposed to the preparing fluid it assumes an irregular outline and a curdled form. The sheath appears like semi-fluid, transparent jelly. The medullary sheath or myeline varies very much in thickness and produces great difference in the thickness of the nerve. One can easily note the nodes and internodes of Ranvier and frequently the nucleus of the neuro-lemma imbedded in the medullary sheath. The medullary sheath is very compressible and hence the medullary nerve may appear narrow at one point and quite thick at another, from the irregular accumulation of myeline.

On the immediate observation of the medullary sheath or even 20 minutes after death it assumes various forms and changes, especially from the preparing fluid and as the result of trauma. The outline becomes quite irregular. I did not attempt to demonstrate the medullary sheath markings of Schmidt-Lanterman.

The coagulation or congelation of the medullary sheath which is albuminous or fatty in character produces an infinite variety in the appearance of the medullary or so-called tubular nerves. The appearance of the medullary sheath depends on the effect of the reagent trauma and stage of coagulation. As the coagulation proceeds the external and internal lines (i. e., the medullary sheath and neuro-lemma) separate more and more while the medulla becomes more lumpy with

globular masses in it, until finally the whole medullary sheath becomes transformed into a coarse or fine granular mass whence the nerve tube looks dark.

In very fresh specimens from the peritoneum the medullary sheath shows itself as a distinct line, and its peculiar contour enables one to recognize a medullary nerve at a glance. The medullary sheath imbibes water and becomes much larger after lying some time in acidulated water.

The neuro-lemma, primitive sheath, or sheath of Schwann forms the

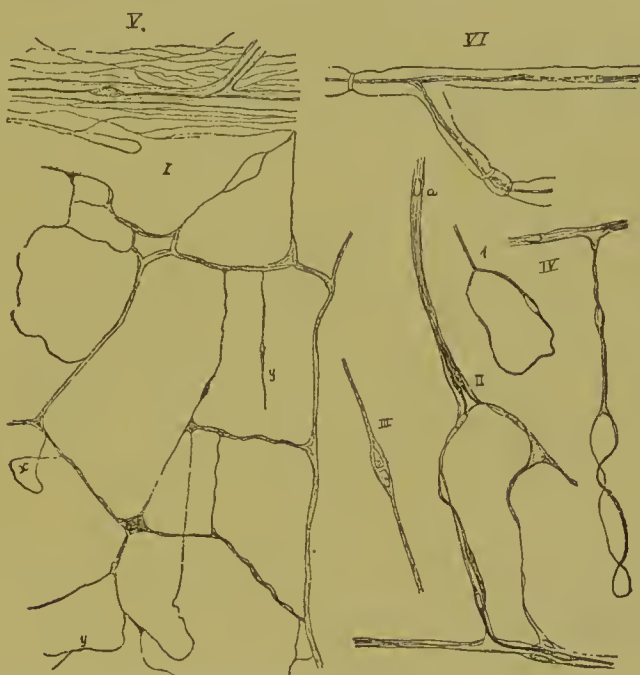


FIG. 181.—(Redrawn, reduced from figures in an article by E. Cyon, 1868.) The nerves were drawn, with mentioned exceptions, from the peritoneum covering the cisterna lymphatica magna of the frog. Gold preparation magnified 200. In this figure y and g show free ending fibres at x, the two free ending fibres make a loop by contact. I shows a distinct net- or mesh-work, but is a network by contact and not from anastomosis. II. A gold preparation magnified 1-500 from the same location as Fig. I. This figure shows spindle-formed swellings. They must be considered as independent bundles of nerves. III. A single nerve fibre with two spindle-formed swellings—nuclei magnified 500 diameters. IV represents fine loops of nerve fibres from the peritoneum covering the cisterna lymphatica magna. A is a gold preparation. B, which is not marked in the figure, but is at the extreme right, is a carmine preparation. V. Fine nuclei bearing nerve fibres from rabbit's omentum. Silver preparation magnified 500. VI. From the mesentery of a guinea-pig. Acetic acid preparation magnified 500.

outer coat of the medullated peritoneal nerve. It is a membrane on which can be observed oval nuclei at various distances. The nuclei correspond to the nodes of Ranvier. The neuro-lemma becomes more apparent as the medullary sheath becomes coagulated and separates from it. One finds it occasionally very difficult if not impossible to demonstrate the neuro-lemma in some of the fine medullated nerves of the peritoneum. In the fresh state when the medullary sheath quite

fills the lumen of the neuro-lemma, one can scarcely discern its outline. In the peritoneum the medullary nerves run in bundles of several to a dozen, or a single nerve courses alone. With gold chloride they are easily recognized by their contour, even among blood vessels, elastic tissue and bundles of connective tissue. The bundle may be composed alone of medullated nerves, but more generally the medullated nerves run single, 2 to 12, in the large bundles of Remak's fibres. The medullary nerves appear to course parallel with Remak's fibres, yet some assume a course which is wavy and thus they cross and recross the Remak's fibres in the same bundle. If the bundle contain only medullary nerve fibres they twist about each other like the strands of a rope. The medullary nerves assume a wavy, sinuous course in the peritoneum. The width of the medullary nerves of the peritoneum varies within wide degrees. Some seem to be three times or more as wide as others. They assume a broad or narrow width. They assume a great length; in fact, the medullary nerves of the peritoneum as far as I can observe are as long as the specimens. This is characteristic of nerve fibres, i. e., they are far-extending nerve cells uninterrupted from beginning to end. As we note the nerves in the muscular portion of the diaphragm under the serosa it may be observed that the medullated nerves may course in unmixed bundles and be of a very narrow type. A whole bundle of medullated nerves, mostly narrow, a few wide ones may be seen without a Remak's fibre or naked axis cylinder being in view. Radiation and lamination are noted to exist in the medullary sheath.

There are two kinds of medullated nerves in the peritoneum, the broad ones and the narrow ones. I found the narrow ones especially numerous in the tendinous portion of the diaphragm of the cat, and the broad ones especially in the mesentery of the cat. Some nerves have a smooth outline but are distinctly in the form of large-bellied spindle forms rapidly succeeding each other, a kind of beaded form. Doubtless there are medullated nerves with the plastic medullary sheath squeezed or forced into smaller and larger accumulations having some relation to the nodes of Ranvier. Where the bundle of nerves makes a sudden curve or kink some of them become ruptured, and out of the ends may be seen the jelly-like coagulated myeline substance of the medullary sheath protruding in various forms of coagulation.

In the broad medullated nerves of the peritoneum we note especially its width and peculiar coagulated or congealed medullary sheath. Irregular fine threads may be noted in its transparent, milky mass and little varied sized globules here and there according to the reagent employed and the time elapsed after death. A recently presented broad medullary nerve of the peritoneum shows a heavy outer dark line and a finer inner



bounding line. These two lines, the "double contour," may not be exactly parallel from irregular coagulation of the myeline. The inner fibre line shows breaks in it from congelation. It soon looks granular or coarsely coagulated. The congelation of the medullary sheath proceeds very irregularly, and hence different segments of the nerve may present quite different aspects. This is due to rapid changes after death and reagents. With the progress of time after death and the application of reagents to the broad medullary nerve of the peritoneum, outer heavier and the inner finer lines gradually but irregularly separate more and more from each other and between them, or even in the center of the nerve coarse granular appearances arise, and globular masses of irregular lines may be observed. The wide medullary nerves

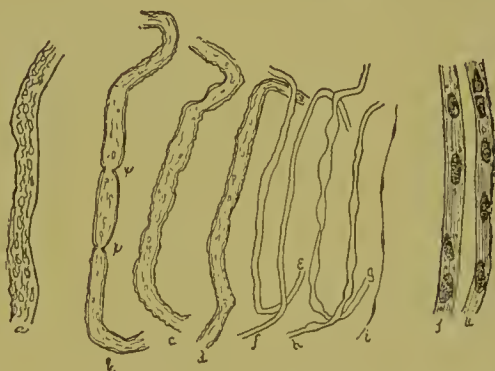


FIG. 182.—(Author.) The individual nerves as they appear under the microscope from the peritoneum after treatment with acetic acid and gold chloride. a, a wide medullated nerve with advanced coagulation, about 45 hours after preparation; b, a wide medullated nerve with two nodes of Ranvier at x and x. A recent and fresh preparation; c, a wide medullated nerve a few hours after preparation. d, a moderately wide medullated nerve shortly after preparation; e, a narrow medullated nerve showing no coagulation; f, a still narrower medullated nerve with the medullary nerve; g, a narrow medullated nerve with the medullary sheath forced into bead-like accumulations; h, a still narrower medullated nerve also showing bead-like accumulations of myeline; i, fibrillated fusiform axis cylinder, non-medullated; j and k, two fibres of Remak showing their nuclei in the neuro-lemma and fibrillated axis. By observing the nerve fibres of the peritoneum from recent preparations and from day to day up to 15 days all kinds and phases of coagulation can be noted.

of the peritoneum may be 1-1500th of an inch (i. e., about 18 micrometers) in diameter.

The narrow medullated nerve of the peritoneum may present entirely different character than its wider relative. It may be only 1-3000 of an inch in diameter (i. e., about 2 micro-millimeters). It may present no double contour, when it is not easy to differentiate it from Remak's fibre. It may be absolutely smooth in contour. It may present a beaded or varicose condition and yet show an even outline. In the "varicose" condition the easily mouldable myeline of the medullary sheath became compressed into irregular masses, inducing an accumulation at one point and diminution at another point. We do not observe the same tendency to lumpy or granular coagulation in the fine,

narrow medullary nerves that we do in the broad medullary nerves. Some are so fine that we cannot detect a double contour. It must be considered, as Frey suggests, that the varicose condition of the fine, narrow medullary nerves of the peritoneum is an artificial one and does not exist in life, and hence must be due to reagents or trauma. It is not always clear what is the cause of the varicose or beaded condition of the fine medullated nerves of the peritoneum, for at times we may note but a single one beaded or varicosed which will run a long distance in the varicosed condition while adjacent nerves remain even in outline. In some portions of the peritoneum the fine medullary nerves course in thick bundles.

3. The nerve fibres of Remak, non-medullated or pale nerve fibres constitute the greater part of the nerves of the mesenteric and omental portion of the peritoneum. Remak's fibres in the peritoneum are quite transparent, faintly striated nerves of varying size. They show nuclei at short intervals. Some consider the fibre of Remak is composed of two elements, viz.: (a) the striated axis cylinder and (b) the nucleated sheath of neuro-lemma. Others consider that the nucleus is simply imbedded in the surface of the nerve fibres themselves. It appears to me that the Remak's band consists of 1, a striated axis cylinder; 2, a nucleated neuro-lemma and 3, more or less medullary sheath. The substance representing the medullary sheath may be very small or even not recognizable. The reason I came to this conclusion is that where certain bundles of nerves of the peritoneum were recently isolated they appeared absolutely like Remak's bands, but as the bundles were kept under observation for some days (7 to 15) in the light (gold chloride preparation) there appeared faint lumpy granulations between the axis cylinder and the neuro-lemma. These observations led me to consider Remak's band in the peritoneum as a nerve consisting of axis cylinder, neuro-lemma and a very slight medullary sheath, and that the nuclei were in the neuro-lemma and not imbedded in the nerve fibres themselves. The axis cylinder appears to be composed of many nerve fibres, sufficient to make a bundle. There is a peculiar branching which belongs to Remak's bands. The branching leads to the formation of a network-like or plexiform condition. This branching, it appears to me, is simply the division or deviation of some of the axis fibres, as many occur in a bundle of medullary nerves. When the fibres of Remak branch off from the original trunk they are entirely covered by the fine, thin neuro-lemma. This entire closing in of the deviating fibres of the Remak trunk by the neuro-lemma lends the appearance that the Remak's bundles divide and anastomose different from the medullary nerves. With this explanation the branching of the medullary and Remak's bundles are exactly similar. The primitive or embryonic condition of nerves is that of no sheath

whatever, the sheath being of connective tissue origin. Some consider Remak's bands as primitive nerve fibres without sheath. But after considerable microscopic labors on the peritoneum with osmic acid, Ag. NO<sub>3</sub>, acetic acid and gold chloride, it appears to me that Remak's bands in the peritoneum in the majority of cases consist of a striated, fibrillated nerve axis and a nucleated neuro-lemma, with a

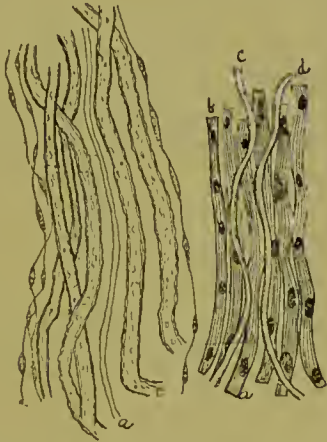


FIG. 183.—(Author.) This is a figure drawn to represent a very frequent occurrence in the nerve relations of the peritoneum. It shows a Remak's bundle (a, b) with two medullated nerves (c, d) coursing almost parallel with Remak's bands. The number of medullated nerves to Remak's nerves in the bundle varies from 2 to 10 as far as I counted, i. e., Remak's bands are 2 to 10 times as numerous as the medullated nerves. Remak's bands are nucleated.

This figure, drawn from a cat's omentum, represents a very common condition found in the peritoneum. It consists of a bundle of medullated fibres. On the lateral edges are one or more strands of non-medullated, nucleated fibres. The medullated nerves are all of the wide variety except one (a), which is of the narrow variety. The nerve strands are in various stages of coagulation and twist about each other like the strands of a rope. The nerves of such a bundle are continuous, uninterrupted from beginning to end. They are broken in the specimen only by the limits of the specimen.



FIG. 184.—(Handbook for Phys. Lab., Vol. II., 1873.) Mesentery of frog prepared in chloride of gold, showing the distribution of non-medullated fibres to a capillary blood-vessel. a, b, A coarse non-medullated nerve fibre giving off finer branches, which forms a plexus round the capillary. Some of these finer fibres belong to the wall of the vessel. (Oc. 4, obj. 8.)

probable trace of medullary sheath. It might surprise a novice in the histology of the peritoneum that one of the most difficult questions I found to decide was whether certain bundles of tissue were composed of Remak's bands or were young connective tissue bundles. It is surprising how the bands of Remak in the peritoneum resemble connective tissue bundles. In some cases I was entirely unable to distinguish



whether a bundle of fibres belonged to Remak's nerve bands or to irregular bundles of connective tissue. The nucleus of the young connective tissue cells often resembles remarkably the nucleus of the neuro-lemma of the band of Remak. Also frequently the resemblance is intensified by the fact that the portion of Remak's fibre between the nuclei on the neuro-lemma is transparent similar to the transparency that exists between two successive tissue nuclei. A distinguishing trait of the bundles which often decides their histologic character is the presence of one or more medullated nerves. There are enormous numbers of Remak's fibres in the peritoneum. In recently prepared specimens of the peritoneum with acetic acid and gold chloride, I have carefully counted the number of Remak's fibres and medullary fibres in a bundle, and it frequently arises that there will be from 2 to 10 times as many Remak's fibres as medullary fibres. At other portions of the peritoneum there will appear bundles of nerves of which every strand is medullated. The single strands of nerves are more apt to be without a sheath—simply the naked nucleated axis cylinder. Not infrequently we can observe large Remak's bundles with two or three medullary nerves, interrupted, constricted at different localities by elastic fibres. The elastic bands resemble a bound sheaf of wheat. The acetic acid induced the Remak bundles to swell, but the elastic fibres would not yield, hence the constriction. The elements which the acetic acid induces to swell must be the connective tissue, the neuroglia tissue and also, perhaps, the tissue composing the neuro-lemma.

In general one cannot draw any conclusions as to function from the thickness or thinness of a nerve unless it be that the thicker a nerve is the longer is its course. Since all embryonic nerve elements consist of nerve cells and naked axis cylinders we must consider all sheaths as evolutionary acquisitions. All nerve sheaths are of connective tissue origin, and serve some purpose not yet fully known. Neither the medullary sheath (the white substance of Schwann) nor the neuro-lemma (the primitive sheath of Schwann) is necessary for a nerve. Neither may cover a nerve in its entire length. Both may enclose the nerve a distance and fail in some other portion. Sometimes one kind of a nerve sheath encloses the axis cylinder and sometimes another. Both sheaths may be absent or both be present. But we must have the axis cylinder and the ganglion cell. So far we are unable to judge the function of a nerve by microscopical observation, no matter whether it be a naked axis fibre, a nerve enclosed in the medullary sheath, or one enclosed in the neuro-lemma.

The distribution of the nerves in the peritoneum are peculiarly irregular. The supply of nerves is rich and abundant. The medullated nerves and fibres of Remak course chiefly in parallel

bundles of two to seventy-five, or even more, in a bundle. The nerve fibres do not run precisely parallel in specimens, but wind and twist about each other like the strands of a rope and the whole bundle takes a wavy or sinuous uninterrupted continuous course. It is probable that the nerves in the original undisturbed bundles are parallel, but the non-parallelism is due to trauma and reagents. The nerves form a mesh-work or net simply by contact and not by real anastomosis. It is a peculiar feature of the nerve bundles of the peritoneum when they deviate or branch off, and the branching strands are liable to course in contact with strands of other bundles. It is difficult to estimate the

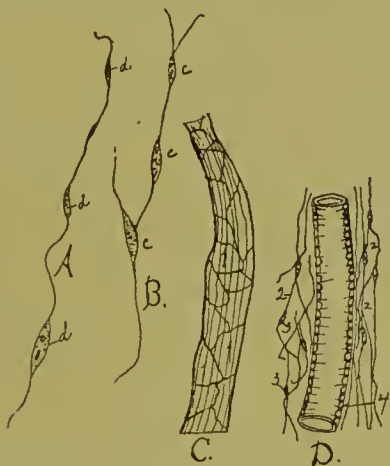


FIG 185.—(Author.) Drawn from a cat's peritoneum to show the method of distribution of nerves. A, A non-medullated nucleated nerve with nuclei at d, d, d. B, A non-medullated nerve with nuclei or swellings at c, c, c. C, A bundle of nerves covered by fine endothelia. D, An artery showing the fine and numerous non-medullated nerves supplying its walls. 2, Points to the fine non-medullated nerves. 3, Indicates its nuclei or swellings of the nerves. 4, Points to the muscular cells of the arterial wall.

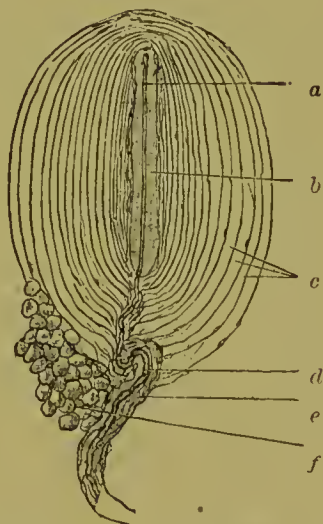


FIG. 186.—(After Stohr, 1894.) A small Vater-Pacinian corpuscle from a cat's mesentery, magnified 50 times. a, Axis cylinder; b, inner beam; c, capsules; d, nerve fibre; e, artery; f, fat cells.

degree of richness of nerve distribution in the peritoneum. In one field of a 300 microscopic diameter I have seen as many as 100 nerve branches, but of course this high number is an exception. In literature I can find very little in regard to the distribution of nerves in the peritoneum. In 1851 Professor Hubert Luschka said he found nerves constantly in the peritoneum. Some few writers quibble in regard to the nerves belonging to the peritoneal membrane or to the subperitoneal tissue. Numerous nerves accompany the subperitoneal vessels, but such nerves really belong to the vessels, and end on the vessels directly. The nerves contain the corpuscles of Vater-Pacini in their course. The Vater-Pacinian corpuscles (cat) are really subserous. The branches

of the subserous nerves are in the serous membrane. L. Jullien observes that the Remak's fibres of the omentum measure two to three micro-millimeters, but may acquire a width of 5 to 6 micro-millimeters.

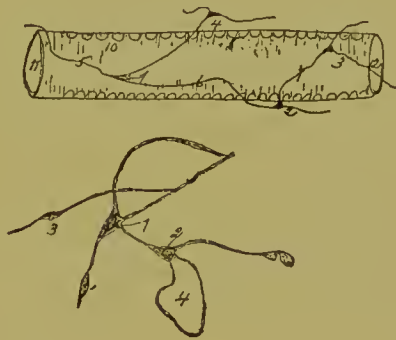
The ending or termination of the nerves of the peritoneum is not fully settled. We have at least two kinds, viz.: (a) the Vater-Pacinian corpuscles, which are terminal bulbs enveloped in numerous concentric capsules of connective tissue. In the cat's mesentery, where they exist abundantly, I have seen 4 corpuscles of various sizes in one microscopical field of 200 diameters. A nerve terminates within the Vater-Pacinian corpuscle. In shape the Vater-Pacinian corpuscle is oval and whitish or opal in color, and varies in size from 1-20th to 1-10th of an inch in length and from 1-20th to 1-40th of an inch in width. The peduncle of the corpuscle consists of a medullated nerve. The corpuscle is attached to the nerve like a berry on a stalk. The peculiar structure which should now be termed the Vater-Pacinian corpuscle was well known to the old German anatomist, Vater, in 1741. About 1830, Pacini of Pisa appears to have rediscovered this corpuscle and given it a systematic description. Since that time Cruveilhier, Henle and Koelliker (Monograph, 1844) deserve especial mention for the study of these wondrous bodies. Excellent and complete papers on the Vater-Pacinian corpuscle may be found in Dr. Merkel's (1880) and Dr. Krause's papers (1884). The Vater-Pacinian bodies are doubtless connected with the sensory nervous apparatus. The concentric laminae of connective tissue must be looked on merely as a capsule. Hoyer claims that each concentric laminae is lined on its surface by endothelia which can be demonstrated by the aid of Ag. NO<sub>3</sub>. The medullated nerve which enters the Vater-Pacinian corpuscle ends in a rounded bulb. The axis fibre travels the whole length of the corpuscle in a non-medullated condition and ends in a bifurcation or button-like knob. The numerous concentric membranous tunics of nucleated connective tissue which enclose the peculiar axis cylinder remind one forcibly of the layers of a large onion. The central axis appears to lie in a transparent homogeneous semi-fluid substance, a kind of water-bed or insulating buffer. The different Vater-Pacinian corpuscles in the cat's mesentery vary wonderfully in size. Some which came under my observation were 3 or 4 times as large as others in the same mesentery. The number of coats enclosing the axis cylinder vary in general from 30 to 60. Some are small, have few tunic coats and resemble Krause's corpuscle. The inner tunics are much closer together than the outer ones. Each lamella or tunic consists of concentric connective tissue and elastic tissue faced by endothelia. The spaces existing between the connective tissue tunics are regarded by histologists as lymph spaces and hence, of course, lined by



endothelia, made manifest by Ag.  $\text{NO}_3$ . The wonderful capsular structure of the Vater-Pacinian corpuscle must be designed to prevent traction, sensory tension or trauma from injuring the delicate axis cylinder and perhaps as a collecting center. The preservation of the delicate axis cylinder of the Vater-Pacinian corpuscle is produced by a fluid medium.

Cyon, in 1868, was one of the first to give an intelligent description of the nerves of the peritoneum covering the lymphatic cisterna magna in amphibia. Louis Jullien continued the work in the omentum majus of man in 1872, and Finkam in 1873 wrote an essay on the same subject. Finkam took the non-pathologic omentum majus from cadavers which came from autopsies in the Goettingen morgue. He used as reagents gold, silver and diluted acid. He claimed that the pale fibres (Remak's bands) were only sparingly present in the omentum majus. Finkam notes, as all observers can plainly see, that the greatest bundles of nerves are in proximity to blood vessels and that when a nerve bundle branches, the

FIG. 187.—(Author.) (Upper.) Drawn from the peritoneal ligament of the gall-bladder of a frog to show a very fine network of nerves ending on a peritoneal blood vessel possessed of considerable muscular tissue. 1, 2, 3, 4, represent nerve ganglia and cells. 5, 7, 7 is the nerve network. 8, 9, 10 show the circular muscles of the artery. 11, 12, the ends of the artery. (Lower.) It shows nerve cells 1, 2, 3. 4 shows a loop of a nerve where the two nerves terminate, running in contact. Note that the nerve cells are quite large.



branch generally runs in the same direction as the original trunk. It may be stated from careful preparations that the nerves of the peritoneum consist of trunks composed of several bundles which run in proximity to vessels. The trunk repeatedly divides, sending out medullated Remak's fibres and non-medullated fibres. On the Remak's bands may be seen at more or less regular distances nuclei on the sheath which are many times longer than the Remak's band is wide. In the course of the non-medullated fibres fusiform swelling may be observed at the greater distance from each other than the nuclei on the sheath (neuro-lemma) of Remak's band. It appears that the swelling on the neuro-lemma of the Remak's band is simply the nucleus brought to view by the gold chloride and the clearing action of acids. It is noticed by every observer that all we can see at the end of some nerves in the peritoneum is a roundish bulb. Is this bulb or end organ related to the fusiform swelling of the non-medullated nerve? At first I thought that the comparison of the serous covering of the cisterna lymphatica

magna of the frog to other portions of the peritoneum might be proper or analogous. For the wall of the lymph sac might be considered of a germinal character, sexual or embryonical state, that it was some persisting part of ancient animal life. Also it might be considered that the nerves were to supply the muscle in the lymph sac. But considerable investigation has convinced me that though each portion of the peritoneum in general has quite an individuality about it, yet the several parts are quite analogous in the nerve supply. However, the fine non-medullated so-called nerve network which Cyon claimed existed in the various regions of the peritoneum is very difficult to demonstrate. Dr. Finkam claims that the end organs of Jullien are simply connective tissue cells.

The other form of the nerve endings or terminations in the peritoneum is more obscure and indefinite. However, it appears from microscopical observation that these nerves end (1,) in connective tissue corpuscles; (2,) around the stomata vera, especially in the cisterna lymphatica magna of the amphibia; (3,) both medullated and non-medullated nerves end directly on the vessel walls; (4,) in single or many celled, capsulated, round or knob-shaped endings; (5,) in some obscure method in the connective tissue not demonstrable by present known methods and reagents. The various shaped and sized bulbous nerve endings are very numerous in the peritoneum.

J. B. Haycroft, E. W. Corlier and Harold C. L. Schofield's reports, accompanied with a distinct figure, show that the fine medullated nerves end by being distributed along the capillary vessel. Dr. W. H. Gaskell asserts that all vascular nerves begin as fine medullated fibres, but lose their medullary sheath in passing through nervous ganglia before they reach their termination in the vessels. The drawing presented by Haycroft, Corlier and Schofield absolutely disproves Gaskell's assertion, for in their "cut" a fine medullary nerve ends on B, the capillary in Fig. 190.

In 1887 F. F. Hoffman made some investigations in the nerves of the peritoneum and concluded that the nerves ended in connective tissue corpuscles and in the protoplasmic cells surrounding stomata vera in the amphibian lymph sac, which is corroborative of my own investigations. In the microscopic investigations of the nerves of the peritoneum a clinical fact is significant and impressive, and that is with the peritoneum about equal in area to the skin with its rich and vast nerve peripheral area, it is not to be wondered that peritonitis produces profound reflex disturbances in the heart, kidneys, lungs and especially the circulatory apparatus. The large number of nerves which may be observed in the peritoneum in one microscopic field of 200 diameters is sufficiently suggestive to the diagnostician to be on his guard for local

and general clinical features from peritoneal disease. The vast network of nerves by contact in the peritoneum allows no locality to escape manifesting irritation. A local point of infection in any portion of the peri-

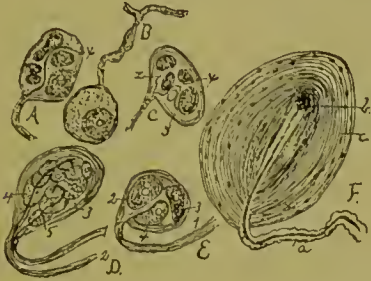


FIG. 188.—(Author.) F, Vater-Pacini corpuscle drawn from the mesentery of a cat. It shows a corpuscle of concentric tunics in which the nerve ends. This is a medium-sized one. Under the microscopic field from which this was drawn there were four Vater-Pacini bodies of various size and shape. b Shows the blunt end of two nerves; c points to the laminated, concentric nucleated tunics resembling the layers of an onion.

E. Peculiar nerve endings in a frog's peritoneum under high power. 1, medullated nerve stalk; 2, 3, large granular cells with bright, round nuclei; 4, the capsule.

D. Drawn from a frog's peritoneum after treatment with acetic acid and gold chloride to illustrate some of the various kinds of nerve endings. 1 and 2 show the entering medullary stalks; 3, the capsule or the end organ containing ovoid granular protoplasmic cells. Under high power. The end nerve organs in the peritoneum, when successfully brought to view by reagents, are very numerous, of varied shape, size and structure. 4 and 5 represent portions of the nerve fibre coursing among the granular cells.

A, B, C. Various forms of nerve endings in a frog's peritoneum from a one celled to many celled. At A one can trace the nerve fibre into the bulb as at x. In B there is one big cell, but no nerve fibre could be traced in the bulb. In C the bulb has many cells, but the dark nerve fibres are plain at z, x and y. Some portions of the frog's peritoneum have scores of these very various bulbous nerve endings.



FIG. 189.—(Author.) This bundle of nerves drawn from a cat's peritoneum represents a very general condition of nerve bundles found in the peritoneum. The main bundle consists, first, of Remak's fibres 1, 2, 3, 4; second, of non-medullated lateral fibres 5, 6, 7, 8; third, of medullated nerve fibres, narrow and wide, 9, 10, 11.

This cut represents a bundle of Remak's fibres from a cat's mesentery. Acted on by acetic acid and gold chloride. In the Remak's bundles there are three medullated nerves, two narrow ones and one wide one. Three bands of elastic fibres are preventing the bundle from expanding. 1, 2, 3, Remak's bands nucleated; 4, 5, 6, 7, are two narrow medullated nerves; 8 and 9 are wide medullated nerves.

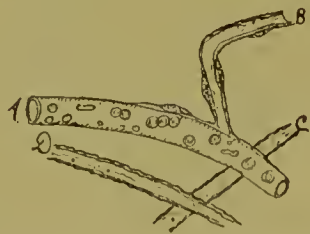


FIG. 190.—(After Hayeroft, Corlier and Schofield, reduced.) A medullary nerve ending directly on a capillary. A, Blood-vessel containing corpuscles; B, fine medullated nerve ending on the blood-vessel, A, directly. C, D, medullated nerves.

toneum will produce local or general reflex intestinal paralysis of the most intense type. The network of nerves in the peritoneum by contact holds the whole peritoneum in an intimate and delicate balance. Under the microscope one can see in well prepared specimens enormous



numbers of non-medullated nerves darting in various directions with the numerous fusiform enlargements which are 3 to 5 times as large as the axis fibre. The swelling is doubtless a nerve cell or ganglion situated within the axial fibre strands. Massive bundles of Remak's bands and large bundles of medullated fibres traverse the peritoneum in various directions.

The vast peritoneal network, by contact, is known by its peculiar shape, different from vessels, blood or lymph, by the peculiar triangular cell, situated at a point of conveyance or divergence of the fibrillated axial fibre. Here and there in the fibrillated axial cylinder we may note the fusiform swelling or nerve cell. Also an accumulation of granular material which seems to separate the axial fibre strands in a similar manner to the fusiform nerve cell situated at more or less regular distances from each other. The network is on a plane with the large bundles of Remak's band and non-medullated bundles, and the most typical nerve network is situated at the most distant point of the nerve bundles. The non-medullated, nucleated, nerve network is seen much better and more distinct in the mesenteries, mesogaster and omenta than in the peritoneum covering the cisterna lymphatic magna. The vast majority of nerves ending on the peritoneal blood vessels are the fine, non-medullated nerves with fusiform swellings on the axis fibre. For a typical place of the nerves perhaps the gall-bladder of the frog is equal to any locality.

The nerves are enormous in number in the peritoneum covering the gall bladder. Doubtless this explains the excruciating pain when calculi become engaged in inducing peristalsis of the duct so highly supplied with nerves. Here many of Remak's bands show distinct anastomosis of the neuro-lemma. Of course, the axial fibres do not anastomose. In the peritoneum covering the gall-bladder one can see the nucleated, non-medullated nerves pass directly to the blood vessel wall and end there.

It may be stated that one cannot assert from observation in the peritoneum whether a nerve be sympathetic or non-sympathetic. It seems that the further that the nerve branches deviate from the main trunk bundles the more liable it is to lose the medulla and neuro-lemma and remain as the primitive naked fusiform axial fibre. The extreme lateral strands of a medullary or Remak's bundle is very liable to become non-medullated and simply present the nucleated axial fibre.

The nerves of the peritoneum present peculiar phenomena in the clinical field. In the first place, the peritoneal nervous periphery is not like that of the skin, though they are regions of about equal area. The skin peripheral nervous apparatus is more sensitive than that of the

peritoneum. However, we know that handling the peritoneum is accompanied by considerable pain; we also know that removing gauze from the peritoneal cavity is accompanied by much pain and distress, yet this may be due to somewhat altered peritoneal nerve apparatus, as is noted when the peritoneum has been inflamed, has deposits on it, or the peritoneum over the bowel has been exposed 24 to 48 hours after colotomy. Such portions of the peritoneum cut with little pain to the patient. The peritoneum does not perceive pain as the skin does, and the effect of disturbance of the peritoneum is pre-eminently shock. The sensitiveness of the peritoneum or its response to irritation is not equal in all regions. Doubtless this is owing to the quantity of nerve supply, and also varies as the irritation is applied to visceral or parietal peritoneal nerves.

The visceral peritoneum is more highly supplied with nerves than the parietal. The sensation of the peritoneum is manifest along the lines of shock, i. e., a profound effect on the vascular apparatus. The magnificent array of nerves supplying the numerous peritoneal blood vessels once started into action by some irritation would profoundly affect the calibre of such vessels and produce immediate reflex or remote effects in the distant organs. The character of the peritoneal pain is that of dullness, periodic in intensity and time.

## CHAPTER VIII.

### THE PHYSIOLOGY OF THE PERITONEUM.

Reading furnishes the mind only with materials of knowledge; it is thinking makes what we read ours.—*John Locke.*

The physiology of the peritoneum demonstrates its utility in the animal economy. In studying its physiology we are attempting to discover what role it plays or what part it assumes in sustaining the normal physical actions of animal life. In determining the function of any organ the first essential is to understand its structure and minute anatomy. The second is by experimentation to observe its natural action, which must include both diseased and healthy conditions.

1. The study of the minute anatomy of the peritoneum discloses the fact that it is not a homogeneous structure, but composed of a varied definitely constituted apparatus. These different structures are, 1, stomata vera, organized canals, mouths, consisting of apertures at the common junction of several endothelial plates and surrounded by granular, polyhedral, protoplasmic, nucleated cells.

2. Stomata spuria, or apertures which exist in the interendothelial space through which connective tissue corpuscles may project or leucocytes may wander.

3. The interendothelial space consisting, when stained with  $\text{Ag. NO}_3$ , of two dark parallel lines whose intervening space is crossed by dark, anastomosing, protoplasmic processes which bind the cells into colonies.

4. Nucleated endothelial cells so joined by their edges as to produce continuous membrane, interrupted only by stomata or interendothelial apertures.

5. Blood vessels.

6. Nerves.

7. Lymph nodes, lymph vessels and interstitial spaces.

8. Various kinds of cells.

The predomination of any one of the above essential elements must decide as to its nature or the class of tissue to which it belongs. The chief and prevailing lymph elements in the peritoneum determine that it belongs to the lymphatic system. The peritoneum is a lymph sac originally and ultimately, though its final form appears quite different from its original. It is so intimately related and connected to many



other organs that it makes its study more complex. However, it must still be studied from its original lymphatic nature. It was originally in development a lymph space, and it is ultimately in function and structure a lymph sac.

We may derive much knowledge from an organ as to its function and utility by its deviations through disease or by experiment. In one case disease experiments with the peritoneum, in the other the experiment is done artificially. In both cases the peritoneum manifests, first, excessive physiology and second, by continuation of excessive physiology we observe a pathologic condition—disease.



FIG. 191.—(Author.) Human omentum majus of boy two years old. Silvered 30 hours after death. It had just become fenestrated (oc. 4, ob. 3, R.) 1, endothelium; 2, 3, stomata vera with granular or guard cells. In 3, near 6, the guard cells have fallen out. 4, 4, 4, Nuclei; 5 and 8, clefts between the endothelia and deposition of debris from the silver salts; 6, intra-endothelia stomata; 7, endothelium; 9, stoma spurium. Note the irregularity of all kinds of stomata. Perhaps 6 represents two leucocytes below the cover-plate.

Again, the structure of the peritoneum in possessing a smooth surface and the physiological function of maintaining it moist and slippery furnishes wonderful facilities for mechanical motion. It produces the maximum motion with the minimum friction. The smooth, slippery surface of the peritoneum, with its wonderful elasticity, allows extensive motion of organs and accommodates itself to rapid or slow distension and contraction of viscera, as gestation and expulsion, dilatation of bowel, stomach and bladder and respiratory movements. However, it is by this very motion that it maintains the circulation of lymph fluids. The motion and structure of the diaphragm makes it at once a suction and a force pump. As the peritoneum originated by the coalescence of

the small lymph or interstitial spaces, due to fluid pressure and independent action of the viscera and body wall, the nature of its structure and function will make it necessary to be classed as a lymphatic organ. Our observations and experiments will be directed to a lymphatic membrane.

As long ago as 1802, when Xavier Bichat fixed his name forever in medical science by his labors on the peritoneum, the name lymphatic, serous or cellular membrane was employed to designate the peritoneum. However, the English authors almost never applied the term cellular to designate the peritoneum. In general the peritoneum is designated by its polish, serosity and by its surface being normally slippery. Organs over which the peritoneum adheres are smooth, while on the surface which the peritoneum does not cover, the posterior surface, it is rough. Where the peritoneum does not cover the posterior surface of the right and left colons, the surface is rough. Where the liver is not covered by the peritoneum it is rough. The uncovered portion of the bladder is rugous. It might be claimed that compression and function make peritoneally faced organs smooth. Bordeu thought that pressure and friction made the viscera smooth on their peritoneal face. This view is not without suspicion, for at whatever age we examine a foetus the peritoneally faced organs are smooth and both peritoneum and viscera equally developed. Function and pressure do not account for peritoneal duplicatures, mesenteries, ligaments and omenta. Motion and pressure do not account for serous membranes, for were that true serous membranes would be developed about large blood vessels which are in continual motion.

If friction, rubbing, be the cause of serous membranes, the peritoneum should be the thickest where the friction is the most active. But the peritoneum is as thick in places of slight motion as it is in places of active motion. It is even thinner over the middle of the ilium, the place of the widest motion, than it is over parts of the parietal wall where slight friction exists.

Friction and mechanical pressure are incapable of producing an organized membrane like the peritoneum. We can, however, say that the wide peritoneal cavity is due to fluid pressure and independent motion of the viscera and abdominal walls. The peritoneum begins and is developed with the viscera.

In regard to the humidity of the peritoneum, it is, like its polish, an inherent quality of its texture. So far as my observations are concerned, the serous fluid in animals' peritoneum is not altered, increased or decreased by digestion or vigorous activity. The transudation and absorption balance each other. If we find some accumulation in the dead peritoneum it may be due to conditions favoring transu-

dation. The fluid contained in the normal peritoneum is albuminous, which facilitates motion, yet produces a certain amount of heat from the friction.

The peritoneum is distinctly foreign in organization to the organs which it covers. Its external surface is mainly adherent to parietes or viscera, yet it may form a band or fold distinctly isolated on both surfaces. The peritoneum is wonderfully adjustable. Organs borrow from its folds and return them as required. The pregnant uterus borrows folds from the ligamenta lata, the vertical colons borrow folds from the vertical mesocola and quit them on contraction of the respective organs. The distending pregnant uterus and well-filled bladder may almost deprive the bladder of all peritoneal covering. The viscera can exist without peritoneum, as may be observed in urachal cysts which strip the viscera of peritoneum. The viscera and peritoneum may be both diseased at the same time, at different times or both separately. The life of the peritoneum, its polish, its lymphatic nature, its humidity, structure and function are separate and distinct from the viscera. Bichat said almost exactly one hundred years ago, "This (peritoneal) absorbing power continues sometimes after death." The peritoneal absorption is more certain if the animal be placed in a warm bath after death so that the animal heat be continued as near normal as possible. Bichat and Cruikshank a century ago disputed Mascagni, who said human bodies would absorb 15, 30 and even 48 hours after death—a subject revived by Von Recklinghausen and K. Ludwig, and recently in a striking manner by Dr. J. H. Hamburger, and which is carefully treated in this chapter by experiments.

Hewson, one hundred and thirty years ago, Bichat, Mascagni, Cruikshank and Hunter almost one hundred years ago considered that the absorbents opened by thousands of mouths on the peritoneal surface. The peritoneum is a great (lymphatic) reservoir existing between the forces of exudation and absorption.

It is doubtful whether the peritoneum is sensitive in the normal animal, at least we can rub the peritoneum and the animal will lie quiet; yet inflamed, no pain is more agonizing. Should the peritoneum be sensitive normally, motion of viscera would doubtless produce disturbances. The tone of the peritoneum is evident from its power to transude, absorb, contract, expand and to resist infectious invasion. The pain in the peritoneum is often a referred pain.

The capacity of the peritoneum to expand depends on its elastic property; on its capacity to unfold its numerous folds or plates; on its capacity of displacement; to shift on its bed and on the adjustability of the interendothelial space. The peritoneum forms a physiologic and anatomic boundary between organs. The different varieties of organs



with their different function are thus circumscribed. The adjustability of the peritoneum allows the vast changes observed in some organs, as the uterus, stomach and bowels, without compromising adjacent ones. The peritoneum induces maximum motion with minimum friction. Visceral motion enhances visceral nutrition. The peritoneum does not act as a mould to shape viscera, because their peritoneal attachments or adhesions are too lax. Again, the peritoneum and viscera quit and re-apply themselves to each other within a wide range. Besides, many of the viscera are but partially covered with the peritoneum. Serous membranes, with the exception of the female peritoneum, represent closed sacs. They cover the adjacent organs like a double nightcap, that is, like a closed sac doubled in on itself.



FIG. 192.—(After Peter Nikolsky, 1880.) Represents endothelia on the small intestine at b, b, while the more rounded endothelia are on each side of the bowel. (a) Is a stoma verum. The contraction and dilatation of the bowel elongates the endothelia over its surface in same manner that it does over contracting and dilating (blood) vessels. Drawn from male frog.

FIG. 193.—(After Peter Nikolsky, 1880.) Represents two stomata vera with endothelia arranged around them in a radiating manner. Drawn from the stomach of a male frog.

Georg Wegner, in 1876, established, by his experiments on animals, a ground-work for the physiology of the peritoneum. He showed that gall, muscle infusion and urine, blood and Na.Cl. solution could be injected into the peritoneum and be absorbed without causing peritonitis. Wegner's labors were epoch-making in the peritoneum and instigated numerous followers to repeat his experiments.

In the physiologic function of the peritoneum we can assert that the endothelial plate, i. e., the cover-plate, assumes a very insignificant role either in absorption or exudates. It may be considered established that

little fluid and no foreign body (leucocyte or microbe) penetrates the cover-plate which is the indurated, metamorphized portion of the protoplasm of the endothelial cell. This leaves for consideration only three distinct structures, to which we must confine the physiologic studies of the peritoneum, viz.: stomata vera, stomata spuria and the interendothelial space. All healthy peritonea contain some fluid which is of an albuminous nature. This fluid is in general of definite amount. Many a time I have poured large quantities of fluid into the abdominal cavity of the dog and noted its rapid absorption and elimination by the kidneys. The peritoneum is automatic in maintaining the proper quantity of fluid, perhaps through pressure. This rapid disappearance of fluids from the peritoneal cavity demonstrates that the peritoneum has vigorous powers of absorption. The varied accumulation of fluid in the peritoneal cavity, known as ascites, demonstrates that the peritoneum possesses the power of exudation or transudation, especially if initiated by an inflammatory process. In general, we may state that all portions of the peritoneum do not absorb fluids universally alike. It has certain localities which possess absorptive powers far in excess of others. Hence, experimentation has been practiced with success to locate the active absorptive areas of the peritoneum. Merely pouring fluid in the dog's abdomen or in the human abdomen, as I have often done in laparotomy, and observing the elimination of the fluid by the kidney does not instruct as to the locality of the peritoneum performing the absorption. The peritoneum is not physiologically equal in function in all parts. We must add some method to the experiment in order to trace the path of the fluid. What are the localities of peritoneal absorption?

To Von Recklinghausen, over a third of a century ago (1862), we must give the credit for the discovery that the diaphragmatic serosa is the chief locality of peritoneal absorption, at least of solid particles. He discovered this fact through experimentation on animals, and by subsequent microscopic observation. Von Recklinghausen found that by injecting finely divided matter suspended in fluid into the abdominal cavity, it would pass through the centrum tendineum of the diaphragm into the subserous lymph vessels, lymph nodes and interstitial spaces of the diaphragm and finally into the thoracic duct. Von Recklinghausen used such substances as milk, Chinese tea, zinnobar, oil, blood, egg yolks, etc. The finely divided matter could be traced into (a) the subserous diaphragmatic lymph vessels and capillaries; (b) into the mediastinal glands and (c) through the thoracic duct, i. e., through the three chief apparatus of the peritoneum. In 1866 Ludwig and Schweigger-Seidel carried on and confirmed Von Recklinghausen's work. In 1871 Auspitz experimented on rabbits by injecting into the abdominal cavity finely divided rice meal. He observed that the rice

grains passed through the diaphragm. He also found that in an hour after injections the rice grains were found in the blood of the ear, and some hours later they were found in the lung, liver, spleen and kidney. Prof. Beck (1893) in an excellent experimental work asserts that granules suspended in fluid injected into the abdominal cavity appear in the thoracic duct one to two hours later. Beck mentions with praise a thesis by Notkins which illustrates the stomata in regard to ascites. So far I have been unable to secure Notkins' essay. The above recorded observations indicate that the diaphragm, or rather that part of the peritoneum known as the centrum tendineum, has special powers of absorption of solids suspended in fluids. Von Recklinghausen poured milk on the diaphragm and observed eddies in the milk stream, and the milk drops disappeared. By allowing Ag.  $\text{NO}_3$  solution to trickle under the cover glass at the point of the eddies in the milk drops, Recklinghausen observed dark spots which he designated as stomata. Notkins injected blood corpuscles into the abdomen, and after a certain time he found the same blood corpuscles in the lymph stream of the thoracic duct which he had so prepared as to watch it. From experiments and observation Von Recklinghausen, Klein, Lawdowsky, Notkins, Nikolsky and others have figured stomata as the points where the absorption occurs, and especially on the diaphragm. Beck reports that Notkins illustrates stomata definitely lined with endothelial cells. Beck claims, in his labors, that by following some suggestions of Notkins and staining the diaphragm with Ag.  $\text{NO}_3$  that he received pictures which dissolved all doubt as to the connection of the peritoneal cavity and subserous lymph vessels. He asserts that the peritoneal cavity and the subserous lymph spaces are directly connected by short canals lined by endothelial cells.

The stomata are the origin of the subserous lymph vessels. Beck allots himself the task of proving the last proposition by injecting into the peritoneal cavity of animals insoluble, finely divided matter suspended in fluid, as oil, blood of dogs and rabbits, zinnobar, etc., and subsequently killing the animal in two hours to four days. The diaphragm was stained with Ag.  $\text{NO}_3$ . From these experiments Beck asserts that stomata can be diagnosed with certainty. The stomata he found partially or wholly filled with the colored granules, and the colored granules gradually spread into lymph vessels from the stomata. Beck is positive in his assertions that his experiments demonstrated that there were distinct and definite anatomical structures—stomata—and that the stomata connected the peritoneal cavity to the subserous lymph spaces by organized channels, and that through these channels or stomata the injected matter found its route from the peritoneal cavity to the sub-diaphragmatic lymph beds. He asserts the above proposi-



tions for the diaphragm only. He makes no assertions in regard to other portions of peritoneum having any absorbing power. The astonishing feature to all beginners is the localizing of absorption of granules suspended in fluid to the diaphragmatic serosa. Previous to this time (1880) investigators began to search the peritoneum for other localities of absorption. In 1882 Dnbar and Remy, in the *Journ. de la Anat. de la Phys.*, claimed that other localities of the peritoneum absorbed than the diaphragmatic serosa, or rather the centrum tendineum. They make two propositions:

1. The absorption of fine granules for the lymph system of the peritoneum exclusively takes place through the diaphragm (centrum tendineum).



FIG. 194.—(Thoma, 1896.) Mesentery of dog. At (a) haemorrhage by diapedesis is going on. At (b) ecchymosis exist from diapedesis, but the aperture in the blood vessel wall is closed again. Leucocytes have emigrated at the same time. X250.

2. The absorption of fine granules from the peritoneal cavity into the blood current occurs through the tributaries of the portal vein. Others have reported careful experiments, as Muscatello, in regard to the absorption of the peritoneal glands. In 1882 the Italian, Maffucci, reported the labor of much experimental work, for which I am indebted to Muscatello's article. Maffucci asserted that the portions of the peritoneum outside the diaphragm which would absorb finely divided granules suspended in fluid are the omentum majus, the ligamentum latum, the ligamentum gastro-hepatieum and gastro-splenicum, Douglas fold, meso-rectum and exceptionally the mesenterium. Maffucci in-

jected Chinese ink suspended in fluid into the abdominal cavity of dogs for his experiments. He killed the animals 1, 6, 24 and 90 hours after the injection. He then examined the lymph glands, the liver, spleen and various places of the serosa.

In the labors of Maffucci he seems to have chiefly chosen the animal after six hours of experimental injection. In the labors of Muscatello, which I wish to discuss for the purpose of noting the absorption by peritoneal glands, the animal was experimented on and then killed even a few minutes later, when the rapidity of absorption and the quantity absorbed was observed. Ellenberger asserts that the dog has a limited lymph vascular system, and for this reason Muscatello employed the dog in his experiments. If any animal has a limited vascular system in experiments there will be more certainty in results, for there will be a less number of paths of absorption, and also a less number of vessels to examine. In my own experiments I found the rabbit the cheapest and most accessible. The experiments on the peritoneum as regards absorption must be often repeated to really understand the mechanism and to be able to recognize the complicated changes observed. Muscatello, after using several kinds of finely divided colored granules suspended in fluid for abdominal injections, finally chose carmine as the most suitable for subsequent microscopic observations. In my experiment I discarded carmine for Berlin blue, because carmine granules in the lymph vessels and on the endothelial surface may be sometimes confounded with blood debris.

No colored matter should be used for abdominal injections which is liable to be confused with any natural product, as the fine black pigment or blood debris, etc. The fluid containing the finely divided coloring matter to be injected into the abdomen should contain .6 per cent. Na.Cl. (physiological) and be of the temperature of the experimented animal. The quantity of fluid used for injection may be according to desired results. I used generally 4 to 8 drams for a four-pound animal. Experiments seemed to prove that clearer physiologic results would be observed by the lesser quantity of fluid. The fluid was injected into the animal in the linea alba. One requires to use care that the end of the needle does not traumatize the abdominal viscera as the animal struggles. The shock to the animal by the injected fluid must not be overlooked, for it produces pain, and so far as I am able to observe, induces pathologic processes in the peritoneum inside of ten hours. The endothelial surface becomes cloudy, the cover-plate softens and loosens, and the exudate thickens from serous-like fluid to thick, tenacious semi-solid material. Hence it would appear from my experiments on the peritoneum of the rabbit, the guinea-pig and the dog that the most appropriate time to observe active physiologic changes, and especially

to observe the action of the leucocytes, is less than eight hours after the injection. If the fluid remains longer in contact with the peritoneum, distinct local pathologic processes become manifest, or better perhaps, physiologic action becomes disturbed. On opening the peritoneal cavity certain macroscopic peculiarities may be observed. One characteristic is that in general the colored fluid tends toward the diaphragm. Another is that the fluid and colored granules are very liable to be entangled with the omentum majus and tend to move toward the diaphragm. Sometimes all or nearly all the fluid was absorbed. Also that the naked eye can detect the localities of absorption in the centrum tendineum or the intertendinous spaces. But to obtain the results of a few minutes' absorption, I am indebted to the labor of Muscatello. My experiments had a duration of 10 minutes and upward. He found by injecting into the abdomen fluid containing fine granules of carmine that it passed through the diaphragm and became deposited in the retro-sternal and mediastinal lymph glands in five to seven minutes. Besides, by lowering the diaphragm and elevating the posterior portion of the animal, the fluid passed through the diaphragm much more rapidly, indicating that the weight of the fluid and its contact with the diaphragm aided in sending it through the centrum tendineum. If the animal were placed on its hind feet and the fluid injected into the pelvic region of the peritoneum, the diaphragmatic absorption would be slower than with the posterior portion of the animal elevated. If an animal be killed a few minutes after the injection of colored solution in the peritoneal cavity, it is found that anterior and posterior thoracic lymph glands and the diaphragmatic lymph vessels contain deposits of colored granules, but the abdominal lymph glands and viscera do not contain a trace of the colored granules. This experiment demonstrates the preponderance of diaphragmatic absorption over other peritoneal localities. In six to eight hours after injection of a solution of Berlin blue with sufficient alcohol to hold it in finest suspension, the diaphragm shows distinctly to the naked eye that it is well filled with the Berlin blue granules. But I found later that it could be well filled in one hour or less. The radiating intertendinous spaces which extend from the muscular posterior point of insertion of the diaphragm to the vertebral column are filled. The chief parts of the diaphragm which the experiment demonstrated as filling with the fluid are the centrum tendineum and the zona teudinea, which can be observed macroscopically. The tendinous spaces and capillaries gradually widen toward the periphery of the diaphragm and finally pass into large trunks of lymphatic vessels, part of which accompany the internal mammary veins, and part of which pass dorsalward in a post-mediastinal course. As the intertendinous spaces pass outward to the lymph trunks, at varying inter-



vals there jut out at right angles club-shaped lymph vessels, sinuses, and spaces like inlets or bays of water. This lateral bulging or expansion of the intertendinous lymph spaces is very extensive in some cases, and in them will frequently be found colored granules because perhaps the slowed stream allows them to deposit.

From these experiments we are warranted in the following conclusions:

1. The primary path by which fluids pass from the peritoneum into the circulation is by way of the lymphatics. The secondary path is the blood vessels. Hamberger, with some others, asserts that the path is by way of the blood vessels.

2. A stream of fluid exists in the peritoneum directed toward the diaphragm.

3. The anatomical structure, physiologic function (respiration), of the diaphragm enables it to act like a suction and a force-pump.



FIG. 195.—(J. Arnold.) Silvered outline of the capillary of a frog after a previous free diapedesis. One can observe stomata vera at the common junction of the endothelial plates and stomata spuria in the interendothelial space.

4. In 5 minutes after injecting a solution holding Berlin blue in suspension into the abdomen, the colored granules may be found in the sub-diaphragmatic lymph bed and intra-thoracic glands.

5. So far as my experiments extend the diaphragm is the primary locality of peritoneal absorption of solid granules. Others assert that the rootlets of the portal vein also absorb fluids, but I have not been able to confirm it.

6. The vast function of absorption performed by the diaphragm and the very small part taken in this process by other portions of the peritoneum accounts for the non-fatality in the purulent condition found at the tubal ends and about the appendix, and the ruptured gall-bladder which is circumscribed and confined by the ligamentum hepato-colicum. The virulent microbes or their products are not absorbed, but circumscribed.

7. On account of vigorous absorptive powers, the nearer peritonitis approaches the diaphragm the more dangerous it is to life.

8. The stomata vera are the most numerous and constant on the diaphragmatic serosa, the mouths of the vast lymph bed located in the diaphragm.

9. When foreign bodies (microbes or colored granules) enter the peritoneum the leucocytes swarm out (a) to digest the invader, (b) to surround or imprison the microbe or (c) to sterilize the germ.

10. It appears to be the leucocyte which carries the colored granules from the peritoneal cavity, through the stomata vera, into the sub-endothelial diaphragmatic lymphatics.

11. The normal peritoneum is automatic in regulating the quantity of fluids it contains. Normally it will absorb all excessive fluid. But in abnormal conditions it may only add to the fluid injected.

12. If potassium ferrocyanide be injected into the peritoneal cavity it will appear in the urine much sooner (about 20 minutes) if the thoracic duct is not ligated. Ligation of the thoracic duct retards its appearance in the urine. Hence, with open lymphatics the ferrocyanide appears in the urine much more rapidly.

13. The diaphragm absorbs fluids perhaps by imbibition. Imbibition is molecular when a mass of tissue absorbs the fluid and capillary when it passes through the pores of the vessels.

14. The diaphragms of dead animals absorb fluid almost as rapidly as those of the living for about a day after death. Absorption of fluids by the diaphragmatic serosa can not be a "vital" process. The diaphragmatic absorption must be a physical property of construction. The perforations in the membrana limitans aid in explaining the matter of rapid absorption. The diaphragm lives perhaps for 3 to 4 hours after death. Is the absorption after death similar to that during life?

The physiologic use of the peritoneum is manifest in rendering every viscus independent in movement of its neighbors, and also of the abdominal wall, i.e., it produces independent movement of body-wall and viscera. The peritoneum also produces fixation of viscera within physiologic limits. However, it is not alone the peritoneum which fixes viscera, for the blood vessels, nerves, mesenteric muscles, atmospheric pressure and the muscular tension of the parietes aid in giving useful visceral support and fixation. The long visceral supports together with the polished, gliding and slipping peritoneal surfaces insure a wide, independent visceral motion. The peritoneal endothelia is not very sensitive, as no pain or sensation of any kind arises when the normal layers glide over each other in the motion of viscera. With the desquamation of the endothelial cover-plates pain arises on motion or friction, due doubtless to peripheral neuritis. In the physiology of the peritoneum one must always bear in mind

the mobility of the peritoneum in certain localities. For example, over large and thick fields of connective tissue one can shift the peritoneum several inches of area. This allows wide mobility of organs with the least chance of trauma, for the nerves and vessels, like the uterine artery, are longer than is required for fixed viscera. However, real visceral ptosis, i.e., steady dragging and elongation of nerves and vessels, causes pain.

The peritoneum may be viewed as a joint cavity lined with synovial membrane. Mobility of the peritoneal joint facilitates the functions of organs and protects them from injury. The peritoneal cavity has many properties in common with joints, as motion, and a thin membrane lubricated by viscid fluid.

In a normal state the peritoneum is quite insensible to pain and motion. Pain is the result of inflammatory congestion and pressure of exudates. The serous fluid, the lubricating oil of the peritoneum, is so small in quantity that one can scarcely determine its composition. It is not a viscid fluid. Robin claims that after the peritoneal fluid has remained some time in the cavity it becomes viscid owing to the presence of coagulable material analogous to mucosine. Bernard claims it contains albumen, sugar, fibrine and coagulates spontaneously. There are found 7 to 8 parts in a 1,000 of mineral elements, almost as in the blood, but the proportions of these elements is not the same. Microscopically in the fluid one observes leucocytes, which may be a little granular, and rarely endothelial cells. In some animals, as the wolf, the white corpuscles are so numerous that it renders the fluid milky. The leucocytes may arise from the blood vessels (diapedesis), the lymph vessels or interstitial spaces. They may again pass out of the cavity. Doubtless the serous fluid of the peritoneal cavity is a constant current, appearing and disappearing all the time. Pathologic processes make the peritoneum manifest in exudation as in ascites, which experimentation of injecting fluid into the peritoneum makes its absorptive process manifest. The absorptive power of the peritoneum far exceeds its exhaling power. It will absorb fluid, gas and solid bodies. Water is the chief and favorable medium to absorb, as it is not irritating to the peritoneum. When fluids are introduced into the peritoneum they become changed by chemical and physical laws, osmosis and filtration.

Ranvier claims that he has observed the leucocytes spread themselves over tissue and become endothelia. Some claim that the leucocytes come from the blood and lymph vessels while Ranvier claims that they have endothelial origin. We ourselves question the endothelial origin of leucocytes, for after considerable physiologic irritation of the peritoneum we can observe large numbers of leucocytes directly under the



endothelia attempting to escape either (mostly at the common junction stomata) of cells or through the interendothelial space. The exhaling power of the peritoneum does not appear to equal its absorptive powers, but we are poor in standards of such measurements. For example, one of our experiments of a dog of 7 pounds absorbed 3 oz. in 15 minutes. Yet the peritoneum has selective qualities for different fluids and also



FIG. 196.—(Author.) Gastro-hepatic omentum of rabbit stained with Ag. NO<sub>3</sub> and logwood. Many guarded cells fallen out. 1, 1, 1, 1, Show beautifully clear oval-shaped nuclei brought out by the staining of logwood. They appear larger and smaller, according to the angle of observation. 2, 2, 2, 2, stomata vera, in some of which all the guard or granular cells are fallen out, in others are partially fallen out; none are perfectly retained. 3, Endothelium: 4, 4, very brownly colored granular cells, around which other endothelia are grouped. They are germinal, young or new endothelia, which are multiplying to take the place of dying comrades. 5, 5, 5, Stomata spuria situated on an interendothelial line. I selected this spot in the gastro-hepatic omentum to show the varied shape of the endothelia, and also to show the number of stomata vera in this region. No shiny or transparent spots (intra-endothelial stomata) are shown in the cut. (Oc. 4, Reichert.)

gases. Gas disappears spontaneously from the peritoneal cavity. Oil and gas are absorbed quite slowly by the peritoneum. More interest, however, is attached to the absorption of blood on account of the frequent haemorrhage in the peritoneum. It is stated that defibrinated blood is absorbed more rapidly than blood. But the peritoneum has the ultimate power to absorb all blood constituents completely, and allow no encapsulating with a healthy peritoneum.

If a lymph stream is slowed or assumes the form of a whirl or eddy it allows granules to accumulate, as in the interstitial spaces of the diaphragm. Investigations have shown at the same time that the retro-sternal glands have the colored granules deposited in them. In regard to the lymph glands of the abdominal cavity, which my microscopical researches did not include, I am indebted to Muscatello. It is important to know that six hours after the experiments the colored granules are found extensively deposited in the glands at the hilum of the liver, spleen and in the numerous glands situated along the lesser curvature of the stomach and along the pylorus. But in the mesenterial glands the deposit of granules is quite limited. The liver contains numerous deposits of granules, partly enclosed in wandering cells and partly in the interlobular spaces. In the spleen the colored granules are found enclosed in leucocytes or in the lymph spaces. In the pancreas testicle the colored granules are found in small quantities, enclosed in the leucocytes or lymph spaces approximate to blood vessels. Muscatello's researches agree partially with the more extensive ones of Maffucci, i.e., of six hours after injection. Maffucci, however, asserts that he found no deposits of colored granules in the mesenterial glands, but he confined his researches mainly to the liver and spleen. With increased time after the injection into the abdominal cavity Muscatello reports that the colored granules are found in all the above named organs, and the glands of the auxiliary popliteal spaces. The results of experimental researches show the following in general, viz.:

(a) A few minutes after the injection of colored fluid into the abdominal cavity the colored granules are exclusively found in the intrathoracic glands.

(b) After six hours and later the colored granules are found in the abdominal glands and organs. Maffucci sought in an experimental way to find subordinate absorption localities in the peritoneum.

To prove that other localities than the diaphragmatic serosa absorb is a difficult matter. I would say that after carrying on experiments of injecting colored matter suspended in fluid into the abdominal cavity with tedious microscopic research for over a year I could not conclusively demonstrate that the lymphatics of the peritoneum except the diaphragm and omentum majus (gastro-splenic portion) absorbed the granules. In other words, I could conclusively demonstrate only that the diaphragmatic serosa (the centrum tendineum) directly absorbs the injected matter. It appears to me it is absorbed to some extent by the gastro-splenic omentum, but that is a very insignificant locality of absorption in the peritoneum when compared with the diaphragm. The difficulty of decision lies in the fact that the absorption outside of the diaphragm is slow and very limited. Also the fact that by injecting colored fluid into

the abdominal cavity, in one to one and a half hours the colored granules could be found in the blood of the ear muscle. (Beck and Auspitz.) Hence, it would be impossible to determine whether colored granules reached the abdominal glands by way of the peritoneum or by the general way of the blood. The difficulty of definite decision is also perceived by Werigo's experiment in which he injected carmine granules into the vein of the ear, and in two to four minutes saw that the granules had become deposited in the liver, and at almost the same time had gained the spleen. Again Maffucci asserts that the position of the colored granules in the liver is the same whether they arrive there by way of absorption from the peritoneum or by way of the jugular. Hence, it must be remarked that by any examination of glandular or parenchymatous organs after six hours of injection of colored granules in the abdominal cavity, we cannot decide definitely whether the granules arrived in the peritoneal glands or organs by way of the lymphatic system or by way of the blood current. To demonstrate the way of absorption (by the blood or lymph currents) by which the glands or organs received the granules, Muscatello undertook a series of experiments on dogs, killing them 20, 30, 40 minutes, 1, 1½, 2 and 4 hours after the colored fluid was injected into the abdominal cavity. In the subjects which had the injection from 20 to 60 minutes he noticed a continuous increase in the deposit of the colored granules in the thoracic lymph glands until they were almost filled. Up to the end of one hour he found no deposit of colored granules in the abdominal lymph glands, the liver or spleen. This is a very significant suggestion, especially when by injecting the colored fluid in the abdominal cavity we find that it has passed through the lymph system of the diaphragm into the thoracic glands in 5 to 7 minutes. This suggests that the colored granules pass many times more rapidly by way of the lymph system than by the blood current, at least in defined regions. It also suggests that the colored granules reach the abdominal or peritoneal glands by way of the blood, and also that the diaphragm is first and last the chief locality of the peritoneal absorption. After the colored fluid had remained in the peritoneal cavity 1½ to 2 hours Muscatello found that the colored granules were found in the liver and spleen, either sparsely free or enclosed in leucocytes. After two hours the granules are met in the peritoneal glands at the hilum of the spleen and those of the liver. It appears from all this experimental research that as soon as one meets the colored granules in the abdominal or peritoneal glands, the granules are already deposited in the liver and the spleen. Muscatello also made experiments by fixing the animal on his hind feet so that the diaphragm was entirely above the caudal end of the body. After the colored fluid had remained in the pelvis, as he stood upright, for 1½ hours, he was killed, and no



colored granules were found in the thoracic lymph gland nor in any abdominal organ. This demonstrated that the upright position of the large dog is unfavorable for peritoneal absorption, and also notes that the diaphragmatic serosa serves a distinct role in the absorption of peritoneal fluids. He then injected colored fluid into the peritoneum of a dog and kept him in the upright position for  $5\frac{1}{2}$  hours, when the dog was killed. The intra-thoracic glands contained colored granules, but neither the diaphragmatic lymph vessels nor the retro-sternal lymph trunks contained colored granules which were perceptible to the eye.



FIG. 197.—(Author.) Human omentum of a woman thirty years of age. Dead twenty-four hours. Ag.  $\text{NO}_3$ . (Ob. 3, oc. 4.) The lightly shaded common endothelia and the darkly shaded germinating endothelia which are growing up over the surface require different foci. 1, 1, Common endothelia; 2, 2, germinating endothelia; 3, clumps debris; 4, lymph or capillary sinus with a stoma verum seen at its bottom; 5, stoma verum. This figure shows merely the formation of a lymph sinus in the midst of germinating endothelia. 4 is a vacuolating cell which eventually will end in a lymph vessel.



FIG. 198.—(Author.) Drawn from the mesentery of frog. (Oc. 4. ob. 3, R.) The sketch represents a nodule of germinating endothelial cells elevated above the surface. 1 represents the stalk of the nodule; 2, the growing nodule; 7, one of the quite granular endothelial cells in the top of the nodule; 6, nucleus of common surface endothelial cells; 5, intra-endothelial stomata; 3, common surface endothelia. This stalk (1) of growing, germinating endothelial cells, of a protoplasmic character, is elevated above the surface of the common endothelia. Notice the grouping of cells in the nodule at 6.

All abdominal peritoneal glands, the glands of the pyloric region, those of the hilum, of the liver and spleen, of the pancreas, of the lumbar and pre-aortic glands, as well as the liver and spleen, were free from colored granules. This is a significant result. It explains to some extent how the violent inflammatory and infective processes which occur in the pelvic appendicular region are not fatal; the pelvic and appendicular region of the peritoneum do not absorb readily nor rapidly. The pelvic and psoatic regions of the peritoneum absorb slowly, almost exactly similar to the slow, safe absorption of the pleurae. Rapid, fatal peritonitis arises when the absorptive powers of the diaphragm are brought into action, or

the region of the small intestine. In nearly all of my experiments I noted the violent share which the diaphragmatic serosa played (and to a limited extent the root of the omentum majus). It appears to me that experiments on the peritoneum of animals demonstrate that all early deposit of colored granules gains access to the abdominal (peritoneal) gland by way of the vast lymph bed in the diaphragmatic serosa. Leaving out the root of the omentum majus as a locality of limited absorptive powers, the other portions of the peritoneum have a very limited share in the absorptive process. However, in my experiments, the interendothelial space in some portions of the peritoneum appeared to absorb the granules of Berlin blue to a small extent. I gave up all coloring-matter for injection in the last half of my experiments except Berlin blue, so that I might become more accurate in the observations in one method and its results. The experiments in the peritoneum demonstrate that the lymph glands found in the hilum of the liver or spleen receive the colored granules before they are found in the parenchyma of these organs. Maffucci demonstrated that the lumbar glands were rich in deposit of granules because the lumbar glands collect the lymph from the pelvis. The glands about the pylorus are also rich in deposit of granules because these glands collect the lymph from a large part of the greater curvature of the stomach.

From experiments with colored fluid injected into the peritoneal cavity we deduce two peculiar but connected propositions, viz.:

1. The chief locality of peritoneal absorption is in the diaphragmatic serosa.

2. There appears to be a stream in the peritoneal cavity directed toward the diaphragm.

Muscattello claims that the diaphragm is the single locality of peritoneal absorption of granular material. The motion of the diaphragm or its pumping action must aid in explaining this acquired function of animal life. As the amphibia have no diaphragm, the function of diaphragmatic absorption gradually arose with its development.

The mediastinal or diaphragmatic glands assumed the function of collecting the lymph stream from the peritoneal cavity. It may be here noted that the method by which the peritoneum absorbs the colored fluid is not agreed upon. Muscattello does not believe that the peritoneum possesses organized pores or canals—stomata, whose function by contraction and dilation regulates peritoneal fluid. He assumes that apertures arise in the peritoneum sufficient to allow the fluid, granules or leucocytes to pass, by a process of retraction of the protoplasm of the endothelial cells. For example, at the common junction of several cells the portion of cells which jut into this common centre retract to form an irregular aperture which resembles the *stoma verum* of Klein,

Lawdowsky and Oedmansson and the absorptive processes occur through this aperture made by contraction. However, I cannot agree with Muscatello, as my labor seems to me to demonstrate stomata vera, i. e., organized canals which connect the peritoneal cavity with the subserous lymph system. However, it must be stated that the investigators who assert and deny stomata vera are about equally divided. It might be asked why the diaphragmatic serosa is the locality of peritoneal absorption and hence has a fluid stream directed toward it. The first of the above questions involves the second.

In 1874, the keen Italian investigator, Bizzozero, gave the only answer so far admitted to be reasonable. He claimed that the membrana limitans is perforated in the diaphragmatic serosa, while in other localities it is continuous. It may be remembered that the free peritoneal endothelia rest on a finely granular, transparent membrane, called by Bizzozero the membrana limitans. This membrane appears to me to resemble ground glass more than any other object. It was observed fifty years ago by Todd and Bowman and named by them "basement membrane." Henle clearly observed it, Arnold, Luschka, Goodsir and others noted it and gave it various appellations. But to Bizzozero was left the discovery that the membrana limitans is perforated in the serosa of the centrum tendineum and zona tendinea. This gives a physical and physiologic reason why the chief locality of the peritoneal absorption occurs at the diaphragm. I have made considerable research on the membrana limitans and have carefully examined it in many places of the peritoneum. It is not difficult to recognize the membrane, but I have not been successful in all cases in finding distinctly the diaphragmatic apertures of the membrana limitans. The apertures in the membrana are very various in their size and shape, many are oval or round. They appear to exist chiefly in groups, and are not uniformly distributed either in number or size over the diaphragmatic serosa. Through the apertures of the membrana limitans it is supposed that the diaphragmatic serosa acquires its absorptive capacity. Its pump-like action aids, doubtless, in producing a stream toward it, in the peritoneal cavity. As a feature that demonstrates that the diaphragmatic serosa is a more active locality of absorption than others, I have repeatedly observed in autopsies with lung disease, especially pneumonia, that the peritoneal serosa has extensive inflammatory deposits over it. The inflammation extends from the pleural serosa by way of the intervening lymph beds of the diaphragm to the diaphragmatic peritoneal serosa. Von Recklinghausen demonstrated that in fatal puerperal peritonitis the diaphragmatic serosa was much more violently involved than other portions. Doubtless the vigorous capacity of the diaphragmatic serosa for absorption explains the rapidly fatal septic peritonitis.



The method of injecting the colored fluid into the peritoneum we shall assume to be the physiologic method, or as nearly physiologic as it is possible. In regard to the introduction of the foreign bodies into the peritoneal cavity by way of experiment to elicit and observe physiologic processes, I do not consider it practical and, hence, shall spend no time with the affair. Any foreign body resting against the peritoneal serosa will sooner or later denude the endothelia and set up a pathologic condition. The physiology of any organ can usually be learned by inducing it to perform action as nearly natural as possible. A solid piece of



FIG. 199.—(Author.) Drawn from omentum majus of new-born child. It represents what I shall term a giant (6) endothelial cell surrounded by many small irregular ones. This irregularity I think cannot be the result of trauma, as it was handled with precaution. The giant cell is less stained than the adjacent endothelia. The omentum of this new-born is in a state of wild irregularity as to shape, size, and grouping of endothelia. 1, 2, 3, 4, stomata vera; 5, stomata spuria (adjacent in the microscopic field, not here shown, are other smaller giant endothelial cells surrounded by smaller groups); 6, giant cell; 7, common endothelia.

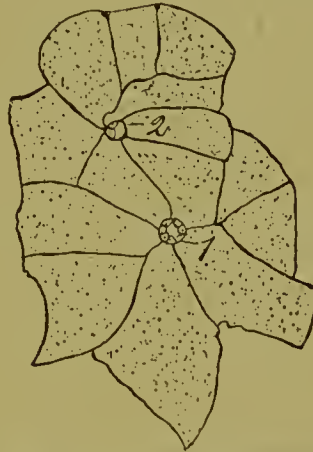


FIG. 200.—(Author.)—Gastro-splenic omentum of two-months' foetal pig (oc. 3, ob. 8 a, R.), showing endothelia grouped around stomata vera. 1, Stoma vera which shows a distinct vertical canal lined with granular cells, which stain well with Ag.  $\text{NO}_3$ ; 2 is not quite so plain, as some of the guard cells have dropped out. The foetal pig shows the most typical grouping of endothelia of all animals which I have examined. The typical endothelia groups surrounding a stoma vera only occur on germinating tracts. It appears that with age the endothelia become more polygonal.

matter chiefly composed of Berlin blue or carmine might be placed in the peritoneal cavity as an experiment, but any of its granules might be transported by numerous swarms of leucocytes which the foreign piece of matter would call out, but transportation by leucocytes borders on pathologic grounds.

In experiments on the liver it has been shown by Maffucci that if colored fluid is injected into the abdominal cavity, the colored granules arrive in the liver by way of the portal vein. The method of experiment was to inject a rabbit of four pounds with an ounce solution, at blood heat, of Berlin blue, suspended in water with sufficient alcohol added

to keep the blue in fine suspension. The rabbits were killed from ten minutes to twenty-six hours after the fluid was injected into the abdominal cavity. The abdomen was opened and the omentum majus, diaphragm and other portions of the peritoneum desired were gently brushed in situ with a toothpick on which was wound cotton batting dipped in the serum of the peritoneal cavity. The brushing to be effectual must be exceedingly delicate, say two to three very gentle, delicate strokes. Too little brushing is not liable to occur, but too much brushing not only desquamates the free endothelia of the peritoneal surface, but disarranges the lymph beds. After careful brushing with all organs in situ, Ag.  $\text{NO}_3$   $\frac{1}{2}$  per cent. solution is gently poured over the brushed surface and allowed to remain some five minutes. All portions of the peritoneum to be examined should now be carefully removed with a pair of forceps and a very sharp pair of scissors, in order to avoid any dragging or trauma. The diaphragm should be removed with especial care by cutting it away from its insertion on the thorax; avoiding if possible smearing it with blood. The parts of the peritoneum which it is desired to examine should be placed in a large capsule of distilled water in the sunlight. The stronger the sunlight, the quicker the specimen becomes brown. After one and one-half to two hours the specimen may be removed to ordinary water, and for final preservation, Muller's fluid, formaline or alcohol are good in the order named. To begin to examine, snip out bits of the thin portion of the diaphragm and place them on the slide, place a drop of glycerine on a cover glass and apply it to the specimen. Two kinds of diaphragmatic peritoneal serosa will be now observed, viz.: one with the peritoneal endothelia intact, and one with it brushed off exposing the lymph vessels. First, the specimen with the peritoneal serosa intact we observe particles of Berlin blue scattered here and there on it; most of the particles or granules are surrounded with white blood corpuscles or leucocytes. Some leucocytes contain a single granule or several of Berlin blue. Particles of Berlin blue too large for one leucocyte are surrounded by many. Leucocytes swarm over the surface when the particles of Berlin blue are distributed. The number of leucocytes appears to be rapidly on hand in accordance to the amount of irritation which the colored granules create. In places large masses are seen which are simply immense numbers of leucocytes collected. Unless the colored granules are very thick and large, there is generally sufficient leucocytes to enclose them. By changing the specimen to one with the peritoneal endothelia brushed off exposing the subserous lymph vessels, we easily discover the Berlin blue granules inside the diaphragmatic vessels in two states, one free and other enclosed in leucocytes. It is easy to see the leucocytes in various situations in the vessel with one or more colored granules in their interior.

Some large granules too large for one leucocyte to enclose are surrounded by several leucocytes. The leucocytes have transported the colored granules from the peritoneal cavity, through the stomata vera, into the diaphragmatic lymph vessels. Often I found (especially in specimens of the guinea-pig) particles of Berlin blue absolutely free in the lymph vessels of the diaphragm. These may have slipped through a stoma verum free into a subserous lymph vessel carried on by the stream of fluid tending toward the diaphragm, or they may have been carried through the stoma verum by leucocytes, and subsequent to arriving in a diaphragmatic lymph vessel become free. With the naked eye we can see that the Berlin blue is deposited in radiating blue lines in between the diaphragmatic tendons. With a microscope we observe that these radiating blue lines are simply intertendinous lymphatic vessels filled with Berlin blue granules. Lateral expansions, bay-like bulgings, jutting out at right angles existing along the intertendinous diaphragmatic lymph vessels in which, on account of the slowing of the lymph current, allows the Berlin blue granules to collect. It may be stated that the particles of Berlin blue found in the sub-diaphragmatic lymph vessels are several times larger than a red blood corpuscle. We noted in these experiments that the leucocytes were found whenever the colored granules existed both on the free surface and the peritoneum, especially in the region of the diaphragm and omentum majus and in the sub-diaphragmatic lymph vessels.

The question of much interest to us is: What is the mechanism of the peritoneal absorption? Having previously decided by experiment that the individual localities of the peritoneum differ widely in absorptive powers, we can confine our labor to the localities of active absorption, especially the centrum tendineum of the diaphragm. From the days (1861) of Von Recklinghausen's famous experiments in which he saw the fat globules of milk forming eddies and disappearing through the diaphragmatic serosa, great interest has been attached to its study, however, only by a few observers. When Von Recklinghausen found where an eddy or whirl was created in the milk and oil globules, and he had watched fat globules disappear, he allowed a solution of Ag. NO<sub>3</sub> to percolate or trickle over this exact spot, and to his surprise the point of disappearance of the fat globules marked a stoma verum, i. e., was located at the common junction of several endothelial cells. It was surrounded by granular, polyhedral, nucleated cells which became much darker than the adjacent cells, perhaps on account of possessing young protoplasm and of possession of considerable precipitable albumen. Now it is through this structure known as a stoma verum that we chiefly base the mechanism of peritoneal absorption.



The stoma verum, on account of the contractive and expansive power of the young protoplasmic cells which line its interior, can enlarge and constrict its lumen. In other words, it has a sphincter at its mouth. It is a physiologic sphincter subject to irritation to dictate its automatic power. The stoma verum can regulate peritoneal currents, and the amount of fluid the peritoneum should contain when inflamed, as in ascites, the vertical canal—the stoma verum—is constricted by the swelling of the polyhedral, granular cells which line it, and hence the ascitic fluid cannot return. When violently inflamed, as in so-called dry peritonitis, the stoma verum being almost closed either by an irri-



FIG. 201.—(Author.) Drawn from edge of human ligamentum suspensorium hepatis. 1, intra-endothelial stomata; 2, endothelium; 3, points to a very large endothelial cell. The grouping of smaller endothelia around a large one, as at 3, is common in the above ligament. 11, endothelia jut against cell 3. Note the peculiar rounded corners of the above cells.



FIG 202.—(Author.) Sheep's omentum majus. (Oc. 4, ob. 3, R.) Some of the granular cells contain nuclei. The figure represents two stomata vera surrounded by somewhat irregular, coarse, granular endothelia. Stoma verum 1 has nine granular cells around its open mouth, while 2 has four granular cells around its closed mouth. The granular cells 3, 4, and 5 contain double nuclei showing rapid growth or division. Some stomata vera show an elongated mouth closed like the human lips, and may have a dozen granular cells lining the circumference of the mouth. 6 and 7 are common surface endothelia and 8 is a nucleus of same.

tated sphincter or swollen cells which line its lumen, fluids cannot pass into the peritoneal cavity. It is the mechanism or function of the stoma verum to which we must look for the chief physiology of the peritoneum. The stomata vera are very numerous on the diaphragm, many are found on the omentum majus (mesogaster), quite large numbers are found on the mesenterium and, besides, being irregularly distributed over the whole peritoneum. The stomata vera are not only the regulators of the peritoneal fluid (nutrition), but are the source of new endothelia. They are regenerative centers to recruit the ranks of dying comrades. Through these stomata vera it appears to me from peritoneal experiments that the particles of Berlin blue are carried by the peritoneal stream and leucocytes into the sub-diaphragmatic lymphatics.

The main numbers of granules are carried through the stomata vera by leucocytes, but some doubtless pass through free after the stomata vera has been dilated by leucocytes loaded with colored granules. Another view, which began with the experiments of Von Recklinghausen and continued, mentioned by Ludwig, Schweigger-Seidel, Dybkowsky, Lawdowsky, Affannasiew, Klein, Burdon-Sanderson, Muscatello, Kolossow and many other good investigators is that the so-called stomata vera are only the apertures made by the retraction of the protoplasm of several adjacent endothelial cells. At the common junction of several cells the point of the cells which projects to a common center retracts, leaving an irregular aperture. The latest chief advocate of the retraction theory in producing peritoneal apertures (stomata) for physiologic action is Muscatello. The explanation which one investigator (Schweigger-Seidel) gave for the presence of granular polyhedral cells around the mouth of the stoma was that they were the nuclei of the adjacent endothelial cells. This view I have, with others, disproved. Another explanation of the presence of the granular polyhedral cells which line the stomata is that they are new growing endothelia, reproduction centers. This is the common explanation of all who advocate the theory of retraction of the protoplasm of the endothelial cells to produce the aperture recognized as the stoma verum. Another view held is that the aperture—stoma verum—is produced by wandering leucocytes, and that the granular polyhedral cells which surround the mouth of the stoma verum are nothing but leucocytes. Ranvier originated this last view. The argument against the existence of stomata vera as anatomical structures is their irregular number and distribution. Others claim that the appearance of stomata is due to accidents of the reagents during preparation. But with all known care in preparation they will appear, which is sufficient to condemn the theory. Again, in the interendothelial space is situated the stoma spurium which is considered by the general consensus of investigators as a connective tissue corpuscle jutting upward, or a leucocyte. This view arose from Virchow and Oedmansson, and, so far as I am aware, is but little altered in thirty-five years. A few consider the stoma spurium as an accidental matter from reagents in preparation, but I have seen the accident occur so many scores of times in preparation that it must be considered as an anatomical factor of the peritoneum.

Thirdly, besides the stomata vera, the stomata spuria, we have to consider the interendothelial space as a factor in the physiology of the peritoneum. The interendothelial space consists (stained with silver) of two dark parallel lines crossed by dark, transverse anastomotic, protoplasmic processes. The dark parallel lines are the edges of the adjacent cover-plates and the dark transverse processes of protoplasm are

those which hold the endothelial cells into colonies. This interendothelial space can and does expand and contract, and doubtless much physiologic action occurs in its domain.

The stomata spuria may be found anywhere in the interendothelial space except the common junction of endothelial cells, i. e., it is found on the interendothelial line. Thus we have the interendothelial space containing the stomata vera and stomata spuria, in which is the seat of physiology of the peritoneum. The endothelial cover-plate, i. e., the indurated, metamorphized, superficial portion of protoplasm of the endothelial cell perhaps is engaged in peritoneal absorption so little that it may be practically excluded from the physiology of the peritoneum. So far as experiments demonstrate or disease indicates, the physiology of the peritoneum is carried on through the interendothelial space, and it is the physiology of the lymphatic system. A word might be said in regard to the white corpuscles or leucocytes. The leucocytes are the protectors of animal life. They are a body-guard of living tissue. A mere warning of the presence of the enemy in any portion of the body is sufficient to enlist swarms of leucocytes to check advancing infection. Experiment shows that where one leucocyte is called out swarms are apt to follow, i. e., the condition which induces the accumulation of leucocytes seems to increase their number. In fact, a characteristic feature of peritoneal experimentation is the army of leucocytes called up by it. The real utility of the leucocytes to the animal economy is to prevent infectious germs from invading the system. The leucocyte may, 1, digest the invading germ; 2, make it sterile, and 3, check its power by imprisonment (exudate). The leucocyte plays an important role in peritoneal absorption. It appears to be the chief medium by which the colored granule is transported from the peritoneal cavity into the sub-jacent lymph nodes and vessels. A few hours after the injection of a solution of Berlin blue into the peritoneal cavity we can find leucocytes laden with the colored granules, both in the peritoneal cavity and in the sub-diaphragmatic lymph system. As my experiments were from ten minutes to eight hours after the injection, I could find colonies of leucocytes blended together into heaps and masses. The colored granules could be observed distributed through the mass. It appears from experiments that as soon as the colored solution is injected into the abdominal cavity the leucocytes begin to swarm out into the peritoneal cavity and attack the granules. The leucocyte seems to quickly surround the small colored granule and then to begin to travel toward the diaphragm and doubtless aided by the peritoneal stream which is set in that direction. If one leucocyte cannot surround a colored granule on account of size, several other leucocytes come to the rescue, and finally the whole particle is rolled up like a ball with leucocytes plastered all



over its surface. Even this mass may be transported to the sub-diaphragmatic lymph system. Doubtless the colored granule found free in the diaphragmatic lymph channels gained its final position by passing through the diaphragmatic serosa after a mass of one or many leucocytes had opened the passage. The stream of fluid in the peritoneal cavity directed toward the diaphragm would aid in floating it through. The common carriers of the colored granules from the peritoneal cavity to the subperitoneal lymph system are the leucocytes. In the study of the peritoneum we really have three similar endothelia (and their mem-



FIG. 203.—(Author.) A, Drawn from adult human omentum majus. (Oe. 4, ob. 3, R.) It shows, 1, a stoma verum surrounded by six cells; 2, a stoma verum; and 3, a very brown spot on the surface of an endothelial cell, perhaps a rift or precipitated debris; 3, free edge of trabecula. Note the irregularity of the endothelial cells over a field of fat globules; doubtless the irregular growing fat globules account for the irregular shape and size of the endothelia.

B, Drawn from an adjacent trabecula (oc. 4, ob. 3, R.) surrounded by six cells. 1, stoma verum with two long rifts and a round white spot in it; 2, 2, endothelia quite irregular; 3, 3, free edge of trabecula. Note in both A and B that the interendothelial lines extend into the stoma verum and that the granular substance shimmers through.



FIG. 204.—(Author.) From woman of thirty delivered twelve hours, who died of eclampsia and acute peritonitis. Bit of a broad band reaching from uterus to rectum. This sketch was drawn with Ag. NO<sub>3</sub>. (Oe. 4, ob. 3, R.) Stoma verum is perhaps a vacuolated cell, as is also 3; 4, shed endothelia (the whole patch is germinating and very brown); 5, nucleus. It shows that on peritonitic bands endothelia frequently spring up, i. e., connective tissue corpuscles flatten out and assume endothelial functions. The only difference that I have so far noted between endothelia found on an old inflammatory peritoneal band and common original peritoneal endothelia is that the new endothelia found on the old peritoneal band of exudate are generally smaller.

branes) with their functions to observe, viz.: (a) the endothelia of the free peritoneal surface, (b) the endothelia of the blood vascular system, (c) the endothelia of the lymph vascular system.

However, it may be stated that no such typical stomata vera can be found in the blood vascular or lymph vascular endothelia as are found on the cisterna lymphatica magna of the amphibia, yet very similar structures exist in the interendothelial space of the blood and lymph vascular system as are found on the free peritoneal serosa (excepting the amphibian lymph sacs). But in many experiments and long

microscopic study of the peritoneum of amphibia, fishes, aves and mammals, I could not convince myself that the functions and especially the structure of the lymph and blood vascular endothelia are exactly the same as the function and especially structure of the free peritoneal endothelia. If all the three serous membranes of the peritoneum (lymph and blood vascular serosa, with the free peritoneal serosa) had the same function and structure, what was proved of the one would be true of the other two. But they do not differ so widely in function or structure as a first glance would indicate. The blood and lymph vascular and free peritoneal serosa all arose from the middle germ layer—the mesoblast. The free peritoneal and lymph vascular serosa are both lymph vascular serosa, hence, in name and origin the same. Doubtless the cover-plate of the free peritoneal serosa is a product of evolutionary process due to ages of friction. Those who have read the excellent labors of Arnold and Thoma (in Virchow's Archives) on the capillaries might consider a comparison between their endothelial membrane and those of the lymph vascular and free peritoneal endothelial membrane as suggestive. (Also the labors in the same field of Ponfick, Recklinghausen, Hoffmann, Auerbach, Aufrecht and others may be found in Virchow's Archives.)

For example, Thoma observed in the vessels of the mesenterium of dogs that sometimes suddenly a stream of red blood corpuscles would break out of the capillary between two endothelial cells, and after a while the opening would close. Thoma considered that this process limited itself to such places on the blood vessel wall where previously leucocytes had passed through. An ordinary conclusion from such a report would be that the leucocytes may produce the apertures between endothelial plates at will. Right here, then, would be the place to learn what really is the power of a leucocyte to travel. We have no doubt that leucocytes have power to travel, for if the peritoneal serosa is irritated swarms of leucocytes quickly come to combat the invasion. The leucocytes arise partly out of blood vessels, partly out of lymph vessels, and lymph sinuses, interstitial spaces; however, they are quickly on hand in vast numbers to defend an invasion of the peritoneum. Investigators from Lawdowsky, the Russian, to Metchnikoff, the French laborer, have attributed all kinds of motive power to the leucocyte. Arnold's beautiful illustrations show that the leucocyte will travel. But it is safe to say that the leucocyte is intelligent enough to move in the direction of least resistance, which is through the interendothelial space. It cannot be denied that the exit of white blood corpuscles or leucocytes from the lymph or blood vessels is due to the peculiar amoeboid movement. But do the leucocytes pass out of preformed openings in the serous membranes or do they make their own channels? Irritation or inflammation of any of these three endothelial membranes of the peritoneum vastly increases

their emigration. They do not pass through the endothelial plate, but their emigration is confined to the interendothelial space. This arouses the view that their numerous migrations in irritation or inflammation of the endothelial membranes is due to retraction of the protoplasm of the endothelial cell. This retraction of the cell protoplasm would necessarily widen the interendothelial space, automatically preparing an easy method of exit for the leucocyte. In retraction of the endothelial protoplasm the interendothelial space is widened or increased in two ways. First, the bodies of adjacent endothelial cells are separated further from each other; second, the anastomotic protoplasmic processes are attenuated, thinned so that they do not occupy so much space, allowing more room for the leucocytes. By watching irritated or inflamed endothelial'serosa one can frequently observe a leucocyte migrating between the endothelial cells or clamped between the edges of the endothelial plates. Lawdowsky, who investigated the property of the leucocytes very much, asserted that the leucocyte would move one-thirtieth of an inch in two hours through blood on a glass, i.e., with no hindrance or obstruction. But that it would require eight to forty minutes to pass through the wall of blood capillary owing to resistance met in the interendothelial space.

In his studies Lawdowsky attributes wonderful power to the leucocyte, even asserting that it will bore through a red blood corpuscle instead of deviating to pass around it. He gives a tragic description of the onward movement of leucocytes through tissue, building their own channels by innate motive power. Kolossow observed the movement of leucocytes of various animals for two years, but denies any such active power attributed to them by Lawdowsky. In my experiments I never saw any such vigorous activity of leucocytes as Lawdowsky describes. But I know inside of eight hours after the injection of a solution of Berlin blue into the abdominal cavity they swarmed over the diaphragm and omentum majus by the thousands, attempting to surround or transport the colored particles to the subjacent lymph channels. Kolossow observed in the stomach of the turtle (which, by the way, I found the most instructive in peritoneal microscopy) leucocytes with numerous pseudopodia lying in the intercellular spaces, and he claims that where the bodies of the leucocytes lay the intercellular spaces were not only widened, but the anastomotic processes were lessened in number, and many had even disappeared. This report would certainly indicate that the leucocyte aided by its motive power to form its own channels. In other words, the irritation of the leucocyte on the protoplasm of the endothelial cell induces it to retract, thus widening the intercellular space. Arnold and others have shown that in inflammation of capillaries the stigmata and stomata never fail. Kolossow



denies preformed openings in the endothelial serosa. Under this condition the stomata and stigmata must stand in direct relation to the emigration of leucocytes or red blood corpuscles (diapedesis). The interpretation of this phenomenon of rapid emigration of leucocytes, with or without denial of preformed openings in the endothelial serosa, is that increased lateral pressure in blood vessels produces conditions which facilitate emigrations of leucocytes.

Some of the above views of the retraction of the protoplasm of the endothelial cell, so that the interendothelial space can widen and narrow to suit the physiologic processes, have been added to science in recent years. The two latest writers who have added their mite to this

theory are Kolossow of Moscow, Russia, and Muscatello of Turin, Italy. But Von Recklinghausen, Lawdowsky, Beck, Klein and Burdon-Sanderson and many others assert that there are, besides all physiologic retraction of protoplasm, organized channels, stomata vera, which connect the peritoneal cavity directly with the subserous lymph



FIG. 205.—(Author.)—From human broad ligament. Age 39, dead 20 hours. Ag. NO<sub>2</sub>. (Oc. 4, ob. 4, R.) The drawing represents two stomata vera: 1 is open, 2 is closed. The endothelia are quite small and of a fairly uniform shape. This specimen was taken from the peritoneum where it diverged from the lower surface of the Fallopian tube. In this locality the sub-serous lines—fibrous and elastic—are very rich in quantity, making it appear that the peritoneum which loosely surrounds the Fallopian tube is quite thick and strong. The germinal endothelia surrounding the stomata vera are intensely brown.



FIG. 206.—(Author.) Is drawn from omentum majus of new-born child to show grouping of cells around a stomata verum. 1, 2, 3, nuclei; 4, granular cells; 5, endothelium. The group is composed of eight cells.

system. I have studied the subject of the peritoneal endothelia with the microscope in all animals at command for two years almost daily and am convinced that stomata vera do exist, the typical ones occurring on the lymph sacs of amphibia. It is on the diaphragmatic serosa where the stomata vera are so numerous, just exactly at the locality of the greatest absorptive capacity.

If one stains the non-traumatized diaphragm of a rabbit or other animal with silver nitrate, under the microscope there can be observed numerous peculiar structures called stomata situated at the common junction of several endothelial cells. It is exactly at the point of stomata where Von Recklinghausen observed the milk globules to duck under the serosa and disappear. By subsequent examination he found that the milk globules passed into sub-diaphragmatic lymph system. A

solution of Berlin blue injected into the abdominal cavity passes through the diaphragmatic serosa, into the sub-diaphragmatic lymph system and nodes in five minutes. But so far as I can observe and what I can learn from other experiments, this rapid absorption does not occur in other localities of the peritoneum. The claim from my experiments is that the dilatation of the stomata vera on the diaphragmatic serosa is the explanation of the rapid transmission of the solution of Berlin blue into the sub-diaphragmatic lymph system and lymph nodes.

Of course, the claim of the discovery of Bizzozero that the membrana limitans is perforated only under the diaphragmatic serosa and nowhere else in the peritoneum is the explanation offered by adherents of the theory of retraction of the protoplasm of the endothelial cell which allows the colored granules to pass through the widened interendothelial space. It is true that the stomata vera are irregular in number and distribution. But under all care and precautions of preparation the stomata vera appear in the microscopic field so that irregularity of number and distribution must not condemn these as anatomical structures. Bichat introduced an experiment which Muscatello repeated; it is that the mesenterium can be blown up, when it will remain so for hours, indicating that the peritoneum of the mesenterium has no perforations in its membrana limitans.

The opponents of the existence of stomata vera announce them as an accident or incident of preparation due to the reagent or trauma. Affannasiew attempted to prove that stomata were due to imperfect preparatory methods. By taking a cover-glass and placing on it soft gum arabic, and then applying this to an absolutely normal portion of peritoneal serosa, he fixed the endothelia. When the gum arabic was afterward dissolved away the endothelia showed no interruptions of stomata vera or spuria. This experiment he considered demonstrative sufficient to exclude the existence of stomata. It is quite probable that the question cannot be settled so easily. However, we do not really need to combat the idea that stomata vera do not exist, for nearly all observers admit that what is generally recognized as stomata vera do exist, but it is the interpretation thereof that plays the role of polemics. Some observers interpret the stomata vera as regenerative centers, others as points of the endothelial cells which project to a common center, and a few as the nuclei of the adjacent endothelial cells congregated about the aperture of the common junction of the endothelial cells. The theorist of protoplasmic retraction considers that at the common junction of several endothelial cells as the body of the cells retracts it leaves an apparent aperture. The writer, with others, considers the stomata vera situated at the common junction of several endothelial cells as an organized channel, lined with polyhedral granular cells. They connect the peritoneal cavity di-

rectly with the subperitoneal lymph space. They regulate peritoneal (nutrient) fluids and currents. They are reproductive centers to supply dying and worn-out endothelial cells. In inflammation their closure obstructs return flows, and ascites results. The cells which line the stomata vera are very granular, sensitive to irritation and contain much albumen precipitable by  $\text{Ag. NO}_3$ . They are much more brown than the adjacent endothelial cells, and stain dark brown with  $\text{Ag. NO}_3$ . Doubtless they are young, growing cells, which accounts for their vigorous characteristics. The stomata spuria situated in the interendothelial space appear to be simply a connective tissue corpuscle or leucocyte projecting upward between the endothelial cells—for reproduction or to allow a leucocyte to pass into the peritoneal cavity to exercise its life functions, which are to digest, imprison or sterilize microbes.

Experiments have taught that the diaphragmatic serosa is the chief locality of peritoneal absorption and that there is a fluid stream directed toward the diaphragm. This should teach that the peritoneal irrigation is dangerous, for it only floats the germs and pus on toward the diaphragm, the dangerous ground of peritonitis. No localized pus in the abdomen should be irrigated, for it would endanger its being carried on toward the diaphragm. From an examination of various portions of the peritoneum in different localities, it will be found that the essential structure varies but little. It covers like a double nightcap the most varied organs with different shape and function, but it will retain its elementary and essential character. Ages of compression and friction have endowed the peritoneum with a highly polished, lubricated surface to serve the purpose of the highest motion with the least friction. Yet the peritoneum has the highly polished, slippery surface at the earliest observable period in the foetus. The albuminous character of the serous fluid endows it with power to have much motion and little friction. The peritoneum has wonderful power in adapting itself to natural distension and contraction of viscera. As one organ fills, it drags on the adjacent folds of peritoneum and appropriates them. Its elastic elements endow it with power to return to normal after distension without losing its integrity.

We must consider the peritoneum in its physiologic capacity as a lymph space or, preferably, as an interstitial space. In examining amphibia, especially the turtle's peritoneum, one is forcibly impressed by the large lymph spaces in the microscopic field. Blood vessels are unsheathed by wide lymph fields, areas and spaces which are wider than any vessel either of blood or lymph. These interstitial spaces are sometimes very large and sometimes quite small, even in the same species of animals. But in all animals the interstitial spaces of the peritoneum are significantly large. The peritoneum itself is one of these



large interstitial spaces. The size of the interstitial space must depend especially on the amount of lymph fluid present rather than its anatomical limits. The large interstitial spaces of the peritoneum act much like the urinary bladder, having great capacity to adapt itself to large or small amounts of fluid. The wide interstitial spaces are neither lymph capillaries nor lymph vessels, but clefts or cavities in the subperitoneal tissue. No one can study long microscopically on the peritoneum, especially of frogs and turtles, without being profoundly impressed with the vastness of the interstitial spaces of the peritoneum. Before the physiology of the peritoneum can be completed these interstitial spaces must be considered. Blood vessels and nerves are ensheathed and invaginated by them. My attention was first especially

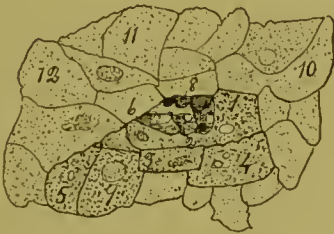


FIG. 207.—(Author.)—Omentum of a woman aged 30. (Oc. 4, ob. 3.) 1, Stoma verum (it is really divided into a light half and a dark half, and both contain nuclei or glistening spots); 2, 3, 4, 5, germinating cells intensely browned with permanent nuclei (notice that besides the stomata vera 6 and 8 there are five other intensely brown cells, no doubt new germinating cells; many similar adjacent fields exist); 10, 11, 12, common flat surface endothelia. The interpretation lies in the stomata vera 6 and 8. It looks as if it was jelly-like, granular protoplasm and was precipitated, aggregated into clumps by the Ag.  $\text{NO}_3$ . In cell 4 the nucleus has two nucleoli.

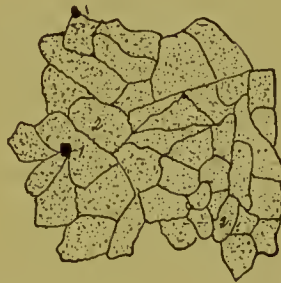


FIG. 208.—(Author.)—Is drawn from human omentum over a field of fat globules. The irregularity of its endothelia is doubtless due to the irregular expansion of the fat globules. 1, 1, Stomata vera; 2, 2, endothelia representing centers of endothelial grouping. Several endothelial cells were required to cover one fat globule, and the microscopic focus required readjusting for endothelia on the top of the fat globule and at its base. In this cut irregularity of endothelial contour and variation of focus for uneven surface are noticeable.

drawn to them when I began to study microscopically the amphibian lymph sacs, and particularly the turtle's peculiar peritoneal structures. What are the functions and significations of these large interstitial spaces in the subperitoneal tissue? Have they any relation to the rapid or slow disappearance of fluid from the peritoneal cavity? Are they merely the roots of the lymph vessels? Are they independent from the blood and lymph vessels? Are they reservoirs of nutrition? The above questions are propounded to indicate the scope of the subject. In the first place, we will consider the interstitial spaces of the peritoneum as an intermediate system between the blood vessels on the one hand and the lymph vessels on the other. They are like a chain of connected lakes, fed by arteries and drained by lymph vessels. The

interstitial clefts or spaces are connected throughout the body. They are the places where the real digestion or nourishment of the tissues is carried on. The richest pabulum of the tissue fluid exists in them. The spaces lie between forces of almost equal pressure, i. e., the feeders and depleters, or supply and waste. The interstitial spaces of the peritoneum under the microscope remind one of vast swamp lands fed by considerable streams (arteries) and drained by apparently slower currents. [The interstitial spaces, the swamp, may nourish vast areas of tissue, as the cranberry marshes nourish vast beds of vegetation.]

The interstitial spaces, the lymph spaces, occupy an intermediate territory between blood vessels, feeders, and lymph vessels, drainers. Their signification is to produce a vast field of a fluid medium in which nourishment and assimilation of tissue elements proceed. The cells sport in the interstitial space of rich nourishing fluid. They engorge themselves with the pabulum and throw off waste material which floats away in the drainage current. It is the fluid medium which is apt in allowing all cell function. The size of the interstitial space is very various. In the amphibia it is simply vast as compared with the blood system. In fact, if we take the view that the interstitial space is for nourishment of tissue, it will be evident that it should be large and extensive so that the wide fields of tissue can be bathed.

The subperitoneal tissue being loose, not compact, will be the typical locality to observe the interstitial spaces, and it will hence be a vast field of real nourishment. It will be the place to study the peritoneal physiology. In studying the peritoneum of a rabbit whose abdomen had been injected fifteen hours before being killed with a solution of Berlin blue, I have sometimes found that the result was pathological, the subperitoneal tissue was oedematous, filled with fluid. The cellular elements were widely asunder and the fluids in the interstitial spaces forcibly distended them. Imbibition had played a role. From the peritoneum of such rabbits the injected fluid had not only disappeared into the subperitoneal interstitial spaces, but the tissue had, doubtless, imbibed more from other regions.

The evidence from these experiments gives us an undoubted key as to where the fluid injected into the peritoneal cavity passes. It passes into the subperitoneal connected interstitial spaces, distending the whole system in all directions. The subperitoneal tissue assumes an oedematous condition. The interstitial spaces contain the fluid that nourishes the tissue as well as their waste products, so that the cell selects and rejects material according to needs and from the fluid in the interstitial space. The fluid in the interstitial spaces is in a state of the most perfect equilibrium of any body fluid, as it is fed from the arteries and drained by the lymph vessels. It is





FIG. 209.—(A. Kolossow, 1895.) Endothelia of the peritoneum on a turtle's stomach. The interendothelial space is drawn to illustrate the anastomotic protoplasmic processes which bind the cells into colonies. In this figure the microscope is so adjusted that one sees deep into the interendothelial space. One can observe the leucocytes in some localities of the interendothelial space with their branches. The specimen was treated with osmic acid and tannin. In this figure the anastomotic processes appear sinuous and not equidistant from each other and of unequal thickness, the superficial being fine and thin while the deeper are thicker and broader.

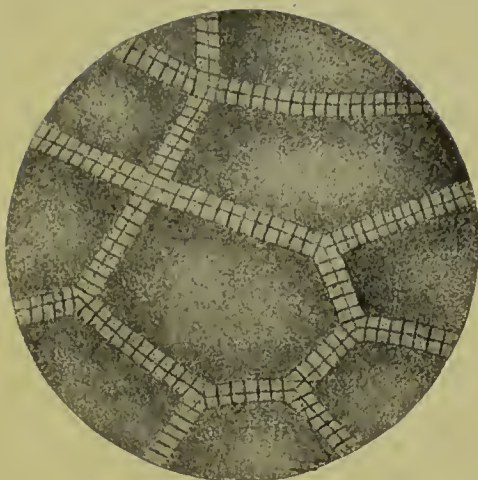


FIG. 210.—(A. Kolossow, 1895.) Endothelia of the peritoneum on the stomach of the oxolotl. Treated with osmic acid (alcoholic watery solution) and tannin. In the interendothelial space one may see the protoplasmic anastomotic processes binding the inferior protoplasmic portion of the cells into colonies. The anastomotic processes are drawn much more uniformly distant from each other than one generally observes in the microscope. The anastomotic processes lie below the adjacent edges of the cover plates. (The microscope is so adjusted as to observe the upper surface of the interendothelial space.)

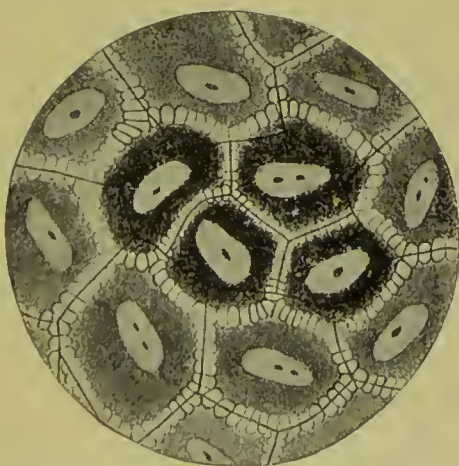


FIG. 211.—(A. Kolossow, 1895.) Represents a group of smaller endothelial, protoplasmic cells among the endothelial cells in the peritoneum on a frog's stomach. Treated with  $\text{Ag. NO}_3$ , osmic acid and tannin.





on the "divide" between supply and drainage. The fluid in the interstitial space of the peritoneum is very quiet, allowing the most perfect condition to bathe and nourish cells. The interstitial spaces are lined by endothelial cells, but the spaces freely communicate with each other. In this vast system of interstitial spaces of the loose subperitoneal tissue float variable quantities of fluid containing food for nourishment and repair of tissue, and also it is laden with all waste products of cell life. This fluid medium, the bread of tissue life, spreads throughout the spaces by various forces, viz.: 1, by diffusion; 2, by mechanical forces, as motion and muscular pressure. In mechanical pressure must be included the heart's activity and the respiratory movements.

The pressure is greater in the interstitial spaces than it is in the lymph capillaries and, hence, the fluid passed into them. The pressure of the fluid in the lymph capillaries is greater than it is in the lymph trunks (thoracic duct) and, hence, the stream flows on in the direction of least resistance. Though all the forces contributing to direct the stream which feeds life's tissues, from the interstitial spaces to the proximal ends of the thoracic duct is not fully understood, yet mechanical forces must not be lost sight of. In the vast interstitial spaces of the subperitoneal tissue lie not only the blood capillaries but the lymph capillaries. The most minute blood or lymph tube is composed of an endothelial membrane whose interendothelial space is provided with an apparatus so constructed (stomata, stigmata) that a liberal interchange of fluid constantly occurs as such tubes course through the interstitial spaces.

In general it may be stated that the fluid from the blood does not pass directly into the lymph capillaries. Fluid from the blood capillaries passes first into an interstitial space and from there into the lymph capillaries. In other words, the interstitial spaces lie between and separate the blood and lymph capillaries. The blood and lymph capillaries are tubular, the interstitial space is irregular and diffuse. The blood and lymph capillaries possess a distinct direction for their streams, but the interstitial space has a mixed, irregular, slow, indefinite movement toward the proximal ends of the thoracic duct. We have three systems to observe, the blood capillaries, a transporter; the interstitial spaces, the place of tissue nourishment; and the lymph capillary, a transporter. I do not think it necessary or conducive to progress to insist that the interstitial spaces are distinctly separate as an independent system, as Adler and Meltzer seem to advocate, but I think it well to impress the idea that the interstitial spaces of the subperitoneal tissue are important factors as nourishing places, as intermediate grounds between the blood and lymph capillaries, as receptacles for fluids, as drains for waste products and as a fluid medium in which cells func-

tionate. So far as I am aware, no experiments have demonstrated that the lymph in the interstitial spaces differs materially from that in the lymph capillaries. The fluid in the lymph spaces must consist of the fluid exuded from the arteries, the waste products of cells, less the fluid used up by the cells in assimilation. It is likely the fluid in the lymph tubes consists of similar fluid. Of course, the fluid in the interstitial spaces must differ according to the kinds of local tissue, according to the kind and degree of waste and repair.

In the previous chapters and the accompanying illustrations we have shown how the canalicular system of Von Recklinghausen, the juice canals of the subserous tissue, are directly connected with the lymph capillaries and the whole with the diaphragmatic lymph bed, so that the subserous tissue is drained into the great diaphragmatic lymph area. Radjewsky demonstrated by experiment that man's diaphragm absorbs exactly similar to that of animals, and by many experiments and microscopic examinations I never could detect any difference in structure between man and other mammals. Diaphragms of the human will absorb carmine 3 days after death, as I proved by experiment. Radjewsky poured the solution with which he experimented on the diaphragm, and the sub-diaphragmatic lymph area was filled in a similar way as it is when the fluid is injected into the abdominal cavity of a living animal. He asserts that one can observe the deposit of the colored granules in the human diaphragm as we can in the experimented animal. He allowed the human diaphragm to absorb for three to twenty-four hours. Hamberger revived the fact, demonstrated by Von Recklinghausen, Ludwig and their pupils, that the diaphragm of dead rabbits would absorb fluids. It may be stated that as Radjewsky found, by using two human diaphragms in the same method of experimentation different results would sometimes occur. We have noted different results in examination of animals, although the method was apparently identical. The explanation of gaining different results in the similar methods of experiments must rest on physical grounds, viz.: the condition of the stomata, the sensitiveness of the diaphragm, the time elapsed after death or the general condition of the living animal, the trauma exercised on it and, possibly, a difference as to the amount of food in the system. If the human diaphragm be cut out of the body with care, and fluid poured over its surface and allowed to remain there for several hours, its sub-diaphragmatic lymph vessels will be found filled with the experimented granules and fluid. I have left the human diaphragm in the colored fluid 8 to 10 hours, but do not think it is the best method as the endothelia are apt to become soft and not clear to examine. An hour or two is sufficient to test its absorptive capacity. The diaphragm should then be stained with  $\text{Ag. NO}_3$ . One can easily



distinguish the blood and lymph capillaries from each other. The lymph capillaries are much broader than the blood capillaries. If there be some narrow vessels their character can be quickly determined by following them to a large trunk. Besides, the lymph capillaries have their well-known irregular calibre, bulgings and contractions and valves. Also in certain specimens one can absolutely observe the lymph vessels pass into the adjacent tissue and blend with the lymph canalicular system, the juice canals, in the connective tissue. Also it is easy to observe the intertendinous lymph channels, which are very various, depending on the separation of the tendinous bundles between which they course. The difference of experimental results in the peritoneum of similar animals doubtless rests on the observation which any one can make in a long study of the lymphatics, and that is the lymph capillaries and small lymph vessels are anatomically in shape, size, and distribution very variable.

In the physiologic study of the peritoneum it may be highly recommended that if a slight irritation or even a slight inflammation be initiated one can observe the elements in a magnified state of activity with much profit. The lymph channels are filled and widened, the subserous tissue become oedematous, forcing apart the cellular elements by fluids displaying the elements in an impressive manner. For example, if one injects a rabbit's abdomen with a solution of Berlin blue and kills the animal fifteen hours later, it will be found that peritonitis has begun and the intertendinous lymph channels are more expanded. To prove that the intertendinous spaces of the diaphragm are really lymph channels one can inject the spaces by the puncture method, when the spaces will dilate to receive the injected matter. Radjewsky practiced the puncture method and found that the injected material not only filled the intertendinous spaces but also forced the material into various offsets and branches of the channels. The various lymph channels situated on each side of the diaphragmatic tendons and between them are connected by vertical or straight tubes through which the lymph is hastened by the pumping action of the diaphragm. The intertendinous lymph vessels of the diaphragm are very manifest on the abdominal side and constitute a vast system of subserous lymph canals. They might be called the lymph system of the tendons or fascia. To condense some propositions we may say:

1. The diaphragm of man and mammals possesses the capacity to absorb fluid, containing finely suspended granules, whence they are deposited rapidly into the diaphragmatic lymph beds and intra-thoracic glands, later becoming widely distributed. The thickness of man's diaphragm makes it difficult to observe. In rabbits one can observe

the process much more definitely on account of the transparency of the centrum tendineum.

2. In slight inflammation all physiologic processes are very much hastened.

3. The desquamation of the endothelial cover-plate would allow much more rapid absorption of peritoneal fluids, but it then becomes pathologic.

Experiments show that, 1, colored granules will pass from the abdom-



FIG. 212.—(Author.)—Horse's gastro-hepatic omentum. (Oc. 4, ob. 3.) Drawn with absolute care. 1, One of the nuclei of a granular cell, i. e., the protoplasm has shrunk; 3, granular cell of stoma verum; 4, rift between two endothelia, debris brushed off; 5, half cells not brushed off; 6, half-cell fallen off; 7, 8, nuclei closed and open (very numerous); 9, a cell around which seven endothelia are grouped intercellularly; 10, rift or precipitate. The drawing is taken from between two tendons and is much lighter in color than the endothelia covering the adjacent tendons. Note the uniform shape and size of endothelia. Large tendinous bundles exist under this endothelium.



FIG. 213.—(Peter Nikolsky, 1880.) Represents a group of germinating endothelia from the dorsal parietal peritoneum of a male frog. Observe that the germinating endothelia are smaller than the adjacent endothelia, that became intensely colored by Ag. NO<sub>3</sub>, and their outlines are more regular than the remainder.

inal cavity into the thoracic duct. 2. Colored material will pass from the blood through the vessel wall into the lymph spaces. 3. Colored granules will pass from the peritoneal cavity into the lymphatics and blood vessels, and finally it will pass into the urine in the bladder. This demonstrates that the walls of the blood vessels, the walls of the lymph vessels and the walls of the lymph spaces (peritoneum) will allow colored matter (and fluid) to pass from one to the other. The passage of the colored material is accomplished through the interendothelial space with constructed apparatus. If colored fluid be injected

into the blood vessels the colored granules will be deposited in the inter-endothelial space of the vascular endothelia. Arnold presents beautiful illustrations of some 500 experiments to show that the colored granular deposit represents not only the interendothelial spaces of blood vessels but of lymph spaces. The colored granules passed out of the blood vessels into the interstitial spaces and became deposited along the intercellular markings. Arnold discovered that by injecting the fluid holding the colored granules in suspension into the blood vessels, peritoneal cavity or lymph sacs, that the colored granules were found deposited in the interendothelial spaces of all three systems, viz.: blood vascular, lymph vascular and peritoneum. Besides, the colored granules passed from the blood vessels into the adjacent tissues and lumen of lymph vessels. Experiments show that fluids are interchangeable in the peritoneum among its blood vascular system, its lymph vascular system and its system of interstitial spaces. The explanation of this interchangeableness must be sought for in the stomata, in infiltration by pressure, by diffusion or by osmosis. Experiments and clinical observation have amply demonstrated that the solid matter passes through the interendothelial space of the three serous membranes, viz., blood vascular, lymph vascular and interstitial spaces (peritoneum). Now, when the fluid is injected into the peritoneum in general, we know it will partially or wholly disappear, occasionally it accumulates. All experiments which I have conducted seem to me to indicate that the primary path by which the fluid leaves the peritoneal cavity is by the route of the lymphatics. In other words, the peritoneal fluid first enters the interstitial spaces. The endothelia which form the lining of the blood vessels, lymph vessels and peritoneum (or interstitial space) are cells that make membranes whose physiology must be sought for in the interendothelial space. The old view of a hypothetic cement substance holding the cells together, I think, must be abandoned and replaced by the transverse, anastomotic, protoplasmic processes, which bind cells into a colony. This view would place the endothelial cells on the same footing as the cells in general. The path by which fluids pass from the peritoneal cavity is doubtless secondarily by the route of the blood vessels. The spanned or relaxed condition of the peritoneum, blood vascular system, or lymph vascular endothelial membrane doubtless influences very much the transmission of fluids. Tympanitis and bowel distension would widen the interendothelial space and thus hasten the passage. The stomata and stigmata under blood pressure, mechanical or muscular pressure might be considered as temporary local expansion of the intercellular space, expanding and contracting according to circumstances. We will introduce at this place some of our own experiments and endeavor to draw some conclusions from their observation.



## SOME EXPERIMENTS ON THE PERITONEUM BY THE AUTHOR.

Experiment No. 1. Rabbit of about 3 lbs. Injected 3 ounce solution 98° F. containing grains of carmine at 5 p. m. Killed next morning at 9 o'clock. All fluids absorbed from peritoneum. No oedema. Many leucocytes found on the centrum tendineum. The quantity absorbed by the peritoneum was 6.4 per cent. of the body weight.

Experiment No. 2. Rabbit 3½ pounds. Injected 3 ounces of fluid with carmine in it at 8 p. m. Killed next morning at 9 o'clock. All peritoneal fluids absorbed. No oedema. Swarms of leucocytes were on the peritoneal surface. Carmine was now discarded for Berlin blue. The quantity absorbed by the peritoneum in fifteen hours was 5.35 per cent. of the body weight.

Experiment No. 3. Rabbit 3 pounds. A 3 ounce solution at blood heat, containing Berlin blue, alcohol and Na.Cl., was injected into the abdomen at 5:30 p.m. Rabbit in much pain shortly after injection. Killed next day at 9 a. m. Perhaps two drams of a cloudy lymph fluid were found in the abdomen. Very slight oedema. Large collections of leucocytes were irregularly deposited on the peritoneal surface, especially on the centrum tendineum. The quantity absorbed was 6.4 per cent. of the body weight in 16½ hours.

Experiment No. 4. Guinea-pig's abdomen. Was injected with a solution containing Berlin blue, Na.Cl., alcohol and water. After 15 hours the animal was killed, and all the fluid was absorbed from the peritoneal cavity. On microscopic examination many leucocytes were found on the serosa. The diaphragmatic lymphatics were found crowded with particles of Berlin blue. The quantity absorbed by the peritoneum in 15 hours was 5 per cent. to 6 per cent. of the body weight.

In the above four experiments, the microscope demonstrated colored granules in the diaphragmatic lymphatics. The centrum tendineum and other parts of the peritoneum were treated with Ag. NO<sub>3</sub> and brushed to remove the endothelia so that the sub-endothelial lymphatics were plainly visible.

Experiment No. 5. Rabbit about 2½ pounds. Injected about 3 ounces of a solution containing H<sub>2</sub>O., C<sub>2</sub> H<sub>6</sub>O., Na.Cl. and Berlin blue into the abdomen and killed the animal 15 hours later. On opening the abdomen all fluids were absorbed. On microscopic examination the diaphragmatic lymphatics were found crowded with Berlin blue. Immense numbers of leucocytes swarmed out on the diaphragmatic serosa. In examination of many parts of the peritoneum, no colored granules could be seen except in the diaphragm. The quantity absorbed by the peritoneum in 15 hours was about 7 per cent. of the body weight.

Experiment No. 6. Rabbit, female. Weight, 700 grammes. With trochar and canula. 50 c. c. m. of water at body temperature was in-

jected into the abdominal cavity. Sufficient Berlin blue was added to make the liquid a light blue color. The liquid was allowed to remain in the cavity forty minutes. The rabbit was killed by a stroke on the back of the head. The rabbit appeared in great pain after the injection and was restless most of the time. On being killed and the abdomen opened by a 2-inch incision, all the fluid was carefully drawn out with a syringe, which was about 3 c. c. m. Allowing a liberal waste while in-



FIG. 214.—(Author.) Gastrosplenic omentum of three-months-old foetal pig. This is a typical group of endothelia surrounding two stomata vera. (Oc. 2, ob. 8a.) 1 points to stomata verum with one cell broken away; it is surrounded by eight endothelial plates; 2, 2, nuclei; 3, 3, endothelial plates; 4, stomata spuria; 5, stomata verum surrounded by plates; 6, 6, stomata spuria; 7, interendothelial stoma; 8, 8, endothelial plates. This very typical figure is drawn as closely as possible to nature. It illustrates the original arrangement of the endothelia of the peritoneum and I cannot too highly recommend the foetal pig for peritoneal study. Its stomata cannot be mistaken and its vertical lymph canals show appreciable depths.

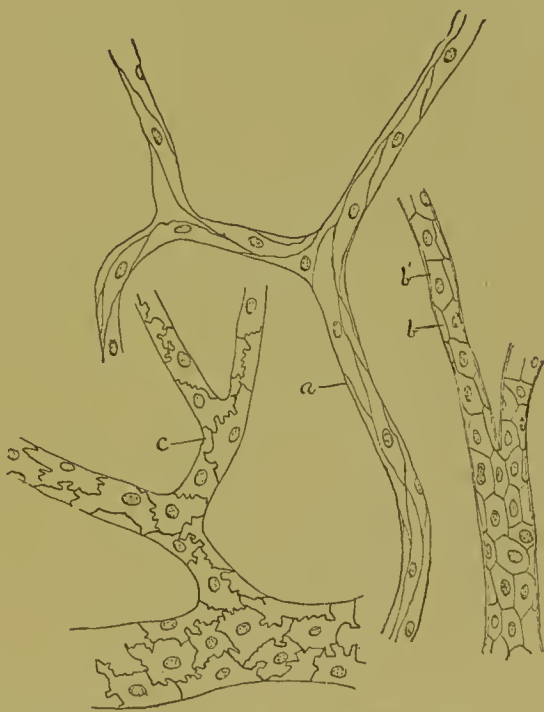


FIG. 215.—(Stricker's Handbook, 1872.) a, Small capillaries with fusiform cells, taken from the mesentery of lenciccus; b, capillaries of the pecten of the eye of the bird, exhibiting polygonal cells; b', hyaloid membrane investing the capillaries; c, capillaries from the intestine of the snail, showing irregularly lobed cells.

jecting and the flowing from the punctured wound as 3 c. c. m., it allows 39 c. c. m. absorbed in forty minutes. With open lymphatics the quantity absorbed while alive in forty minutes was 39 c. c. m., 5.5 per cent. of the body weight.

Experiment No. 7. Same rabbit, dead. Abdomen being opened and all fluids being drawn out of the cavity, we injected 50 c. c. m. of

water at body temperature and closed the abdomen hermetically with double rows of strong suture. After forty minutes abdomen was reopened and the fluid carefully drawn out with a syringe. About 10 c. c. m. of a cloudy appearing fluid were collected. About 39 c. c. m. were absorbed by all the peritoneum of the dead rabbit and that, too, after it had during life absorbed 39 c. c. m. 10 minutes before. So that here it could be estimated that the dead peritoneum absorbed more than the living one. During 90 minutes 78 c. c. m. were absorbed, with 10 minutes' interval of rest. With open lymphatics the quantity absorbed in 40 minutes while dead was 39 c. c. m., 5.5 per cent. of the body weight.

However, we can hardly consider the diaphragm dead until 3 or 4 hours after cessation of heart-beat and respiration. Peritoneal absorption of fluids cannot be considered a vital process, but a purely mechanical process. One should be careful in introducing the fluid, as it is very easy to wound the intestines. One of the best methods is to introduce a trochar and canula, then withdraw the trochar, when the point of the canula can be moved freely about in the abdominal cavity without injury to the viscera. The rabbit lay on its back all the time except  $1\frac{1}{2}$  minutes to kill it and  $1\frac{1}{2}$  minutes to weigh him, for it is known that hanging the animal up by the hind limbs hastens the peritoneal absorption as well as elevating the hips—all hastening the approach of fluids toward the diaphragm, which is the chief locality of absorption. The rabbit's abdomen was not massaged, for that hastens peritoneal absorption and lymph (duct) secretion.

#### MICROSCOPICAL EXAMINATION.

The gastro-splenic omentum and the centrum tendineum were examined. The Berlin blue was found in the intertendinous spaces, often in large clumps. The leucocytes swarmed out on the diaphragmatic surface. Some contained particles of Berlin blue inside of their bodies. The centrum tendineum was oedematous, all fibrous bundles being well and abnormally separated from each other by fluids, i e., the intertendinous lymph spaces were dilated.

The gastro-splenic omentum did not show an especial share in the experiment. No particles of Berlin blue could be found in its lymphatics, nor could oedema be distinctly observed.

There was a defect in this specimen, owing to treatment. The diaphragm was brushed too vigorously and then it was placed in a bottle and shaken too much by a journey. The defect lies in being too much brushed and too much washed. But the deposits of Berlin blue in the intertendinous lymph spaces were distinct.

In 1889 and 1890, in experimenting on dogs to perfect a technique in intestinal surgery, I often poured the dog's abdomen full of warm



water to test its effect on the animal. The only effect I observed was that the dog urinated very frequently and in abnormally large quantities for some 50 hours, so that the disappearance of the fluids from the peritoneum has long been known, from ancient and modern experiments. But the paths of absorption, whether it be by way of the lymphatics or the blood vessels, remains unsettled today.

Experiment No. 8. Rabbit, female; weight 1,000 grammes. Injected 50 c.c.m. of 0.5 per cent. Na.Cl., 2 c.c.m. of a 5 per cent. solution potassium ferrocyanide with a little Berlin blue. About eight minutes later by pressing on the bladder urine trickled out, and by adding ferric chloride a faint blue reaction arose. In twenty minutes after the injection into the abdomen the urine gave a deep blue, and forty minutes after the injection the urine was of a very dark blue by adding the iron and salt. By adding the iron and salt to the urine a fine blue color arises if any  $K_4 Fe Cy_6$  be present, even in extremely small quantities. At the end of forty minutes the rabbit was killed, the abdomen was opened and 8 c.c.m. of fluid drawn out with the syringe. Allowing a liberal waste of 5 c.c.m., it would leave 37 cubic centimeters which the rabbit of a 1,000 grammes absorbed in forty minutes. The quantity absorbed in forty minutes, while alive, was 37 c.c.m., 3.7 per cent. of the body weight.

With free lymphatics fluids pass from the peritoneum into the bladder in eight minutes.

Experiment No. 9. Same rabbit dead. Injected into the abdominal cavity 50 c.c.m. of 5 per cent. Na.Cl. solution at blood heat and sewed up the abdomen with the rabbit lying on his back. In forty minutes I re-opened the abdomen and drew off 20 centimeters with a syringe. Allowing a liberal waste, the dead rabbit's peritoneum absorbed 28 c.c.m. While dead in forty minutes the peritoneum absorbed (28 c.c.m.) 2.8 per cent. of the body weight.

In the preceding experiment observe that a 700 gramme rabbit absorbed more than this 1,000 gramme rabbit, both dead and alive.

#### MICROSCOPICAL EXAMINATION.

The microscope showed that the Berlin blue was thickly and richly deposited in the intertendinous lymph spaces of the diaphragm. One can easily observe the colored granules between the tendons irregularly deposited. Leucocytes can be seen clamped between the borders of the cover-plates emerging from a stoma verum. They can be observed shimmering through the transparent endothelium as they are approaching a stoma verum or spuria. The particles of Berlin blue can be seen numerously deposited in the wide capillary lymph channels or spaces through both the free peritoneal and lymph vascular endothelia, but very

few places can be found where the blue particles can be seen entirely surrounded by the leucocytes; perhaps the time, 40 minutes, was too short for the process to complete itself. But in many places the leucocytes can only be found in contact with the particles of Berlin blue, i. e., partially surrounding it.

Experiment No. 10. Rabbit; weight, 1,050 grammes. Anesthetized chloroform. Left jugular vein ligated. 50 c. c. m. (of  $H_2O$ , 48 parts and 2 c. c. m. of a 5 per cent. solution of potassium ferrocyanide) at blood heat was injected into the peritoneal cavity. He suddenly died

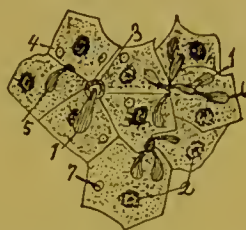


FIG. 216.—(Author.) A figure to represent a portion of the peritoneal serosa of the centrum tendineum of a rabbit after injecting fluid into the peritoneum for forty minutes while alive and again forty minutes while dead. The specimen was slightly silvered, then prepared by a solution of gold chloride 1 part, acetic acid 5 parts and water 994 parts, whence it presented a most beautiful and brilliant picture. 2, Nuclei; 1, shows the leucocytes emerging from the sub-endothelial spaces, through stomata vera or stomata spuria—however always through interendothelial space; 3, points to a leucocyte emerging from a stoma verum; 5, indicates a leucocyte emerging through a stoma spurium; 6, is a leucocyte entirely free. Note that the leucocytes become elongated as they emerge. 4 and 7, point to the leucocytes shimmering through the coverplate. Under the cover-plate the leucocytes are round. Peritoneal irritation induces the leucocytes to come to the surface.

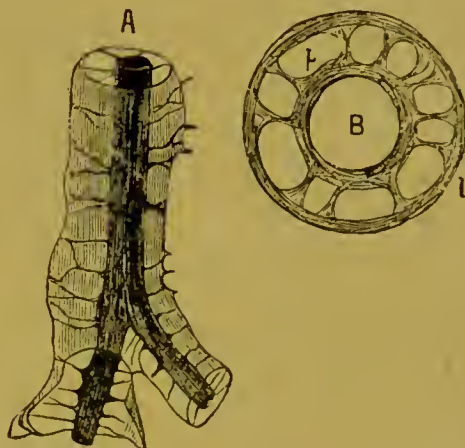


FIG. 217.—(Gegenbaur.) A. Peri-vascular lymphatics of the turtle's aorta. B. Cross-section of an artery from the brain showing peri-vascular lymph spaces divided. It is known as the peri-vascular space of His. The Virchow-Robin's space is the lymph space in the adventitia of blood vessel wall. The turtle is the type of all animals, so far as known to the author, to show typical lymphatic spaces of the peritoneum and peri-vascular spaces.

11 minutes later from the chloroform. Re-collected after opening the abdomen 20 c. c. m.—30 c. c. m. absorbed in 11 minutes.

With lighted lymphatics, while alive, the peritoneum in 11 minutes absorbed (30 c. c. m.) 2.8 per cent. of the body weight.

With ligated lymphatics, while dead the peritoneum in 11 minutes absorbed (28 c. c. m.) 2.6 per cent. of the body weight.

(No reaction in the urine in 11 minutes with ligated lymphatics from the addition of iron salts.)

Experiment No. 11. The same rabbit dead. 50 c. c. m. of  $H_2O$ , at blood heat was now poured into the dead rabbit's abdomen and the cav-

ity closed by sutures. Berlin blue was added to color. In 11 minutes rabbit's abdomen again opened and collected 22 c.c.m., absorbed. 28 c.c.m. The abdominal peritoneum gave a quick reaction to the iron salt after 11 minutes, but no reaction could be gained by introducing cotton into the thoracic cavity wet with iron salt. Hence the solution of ferrocyanide had not gained the bladder or the pleural cavity in 11 minutes, nor even 10 minutes later when it was again tested. With the left jugular vein ligated the cyanide salt had not gained the bladder in 11 minutes, as shown by testing, while with the jugular vein not ligated the cyanide salt gains the bladder in 7 to 8 minutes.

This rabbit of 1,050 grammes showed rapid absorption, absorbing during life, in 11 minutes, 30 c.c.m., and after death 28 c.c.m. in 11 minutes, with the left jugular vein ligated.

Microscopically.—In 11 minutes after death the particles of Berlin blue gained the lumen of the lymph vessels of the sub-endothelia of the diaphragm. They are chiefly found in the interstitial lymph spaces lying between the tendon bundles of the centrum tendineum. They are mainly found along the wall of the large valved lymph trunks, occasionally toward the center inside of a leucocyte, in contact with a leucocyte or free.

Experiment No. 12. Human diaphragm  $2\frac{1}{2}$  years old, male, 36 hours after death absorbs Berlin blue particles by being placed in water with Berlin blue in it. The Berlin blue particles are found between the tendons and more plenty in the large branches of lymph vessels which lie along the circumference of the centrum tendineum. The colored granules are not so richly deposited in the lymph vessels of the dead as in the living, as the pumping action of the dead diaphragm is lost. A diaphragm which absorbs 36 hours after death must depend on some other factor than so-called "vital" action. The process of diaphragmatic absorption must rest on some mechanical principle—perhaps due to the perforations of the membrana limitans, which are almost solely located on the centrum tendineum.

Experiment No. 13. Dog of about 6 lbs. Anesthetized and the left jugular vein ligated. Injected into the peritoneal cavity 100 c.c.m. of fluid ( $H_2$  0.98 c.c.m. and 2 c.c.m. of a 5 per cent. solution of  $K_4FeCy_6$ ). He suddenly died in 11 minutes from the chloroform and the fluid was removed, absorbed 50 c.c.m.

Experiment No. 14. Same dog, dead. Abdomen opened. 100 c.c.m.  $H_2O$  injected (with Berlin blue) and re-collected in 11 minutes. 20 c.c.m. absorbed. In 11 minutes no reaction of the urine by  $Fe_2Cl_6$ .

In the very short experiments the leucocytes do not emerge on the diaphragmatic surface in such swarms as they do in the experiments of longer duration. However, in the two experiments, one of forty min-



utes alive and forty minutes dead, one may observe in specimens treated with this solution (of water and acetic acid 1 to 200 and of this mixture 1,000 parts, and gold chloride 1 part) the most beautiful examples of the leucocytes attempting to emerge through a stoma verum or spurium. Also one can observe the leucocyte on the under side of the cover-plate.

Microscopically.—The Berlin blue gained the diaphragmatic lymphatics in 11 minutes, though dead, without diaphragmatic motion.

With ligated lymphatics while alive, in 11 minutes the peritoneum absorbed (50 c. c. m.) 8.8 per cent. of the body weight.

With ligated lymphatics while dead, in 11 minutes the peritoneum absorbed (20 c. c. m.) 2.2 per cent of the body weight.

Experiment No. 15. Duration of experiment, 30 minutes. Dog weighed about 20 pounds. Chloroformed almost continuous with ligated left innominate vein. Injected 100 c. c. m. into peritoneal cavity ( $H_2O$ . 98 c. c. m. plus 2 c. c. m. of 5 per cent. solution  $K_4FeCy_6$ ). Killed by chloroform in 30 minutes, 80 c. c. m. absorbed. Tests for urine every 5, 10 and 25 minutes, but no reaction of blue in the urine by adding for coloring  $Fe_2Cl_6$ .

No blue color in the urine in 30 minutes with ligated lymphatics.

Experiment No. 16. Same dog, dead. Injected 100 c. c. m.  $H_2O$ . with sufficient Berlin blue for coloring. In 30 minutes 70 c. c. m. absorbed. At the end of one hour and ten minutes after the first injection the urine reacted deep blue, and the peritoneal cavity reacted blue, but the pleurae did not react blue.

Microscopically.—I examined a few specimens only of the diaphragm, but did not secure good points showing the Berlin blue. The diaphragm appeared blue in between the tendons.

With ligated lymphatics while alive, the peritoneum, in 30 minutes, absorbed (80 c. c. m.) 1.06 per cent. of the body weight.

With ligated lymphatics while dead, the peritoneum absorbed in 30 minutes (70 c. c. m.) 0.93 per cent of the body weight.

Experiment No. 17. Rabbit; male. Weight, 953 grammes. Left innominate vein ligated. Injected into abdomen 50 c. c. m. of a 1 per cent. Na.Cl. solution at blood heat (with 2 c. c. m. of a 5 per cent. solution of potassium ferrocyanide with sufficient Berlin blue for coloring). Duration of experiment, 30 minutes. Urine tested every 5 minutes after the first 7 minutes. The first delicate blue reaction occurred at the end of 30 minutes. All fluid collected. Absorbed 8 c. c. m. Rabbit killed with ether.

Experiment No. 18. Same rabbit, dead. Now 50 c. c. m. at blood heat of a 1 per cent. solution of Na.Cl. was injected into the abdomen and the abdomen closed by sutures. At the end of thirty minutes the

fluid was again collected, amounting to 16 c. c. m. Absorbed 34 c. c. m. By close examination after death I found that I had included in the ligation of the left innominate vein the carotid artery, the pneumogastric and the phrenic nerve.

Microscopically.—The Berlin blue which was added to the experiment while the animal was living was found beautifully deposited in the centrum tendineum.

With ligated lymphatics while living, the peritoneum absorbed (8 c. c. m.) in thirty minutes, 0.88 per cent. of the body weight.

With ligated lymphatics while dead, the peritoneum absorbed in 30 minutes (44 c. c. m.) 3.57 per cent. of the body weight.



FIG. 218.—(Klein, 1880.) Two endothelial plates of mesentery of newt, stained with picrocarmine. Each endothelial plate contains a hyaline slightly stained ground plate, a plexus of fine fibre-bundles—intra-cellular network—in connection with the intra-nuclear network.

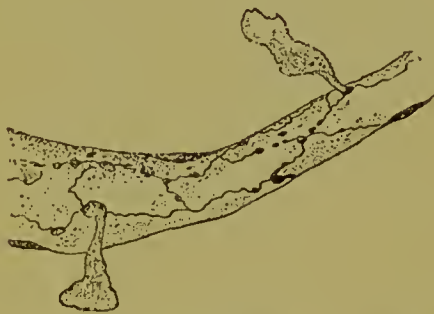


FIG. 219.—(G. Engelmann.) Capillary from the mesentery of dog with two migrating leucocytes. Injected with silver, stained with borax carmine. Note the black dots marking stomata vera and spuria. The plastic condition of leucocytes enables them to pass through small apertures.

The blue color appeared in the urine in 30 minutes, proved by pressing on the bladder, forcing out the urine and adding  $\text{Fe}_2 \text{Cl}_6$ .

Experiment No. 19. Dog, female. Weight, about 7 pounds. Anesthetized with chloroform. Left innominate vein ligated. Injected into the abdomen at blood heat 100 c. c. m. of a solution ( $\text{H}_2 \text{O}$ ., 48 c. c. m., 2 c. c. m. of a 5 per cent. solution of potassium ferrocyanide). In 5, 10 and 15 minutes after the injection the urine was pressed out of the vagina, but gave no reaction to the ferric salt. No reaction could be gained until the animal was killed, 30 minutes after the injection into the peritoneal cavity. At death, the bladder being opened, the urine gave a pale blue reaction. 95 c. c. m. were absorbed in 30 minutes. A blue reaction could be secured over the peritoneal cavity, but none in the pleural cavities.

Experiment No. 20. Same dog, dead. 100 c. c. m. of a watery solution of Berlin blue at blood heat was injected into the peritoneal

cavity and the abdomen was closed with sutures. In 30 minutes 55 c. c. m. was absorbed. No oedema could be observed. The Berlin blue could be seen by the naked eye filling in the intertendinous lymph spaces of the centrum tendineum.

The microscope showed not only the intertendinous spaces loaded with blue particles by the large lymph vessels of the diaphragm, but also the lymph vessels of the gastro-splenic omentum. So far I have not found the blue granules in the mesentery, but we have found the colored granules in the centrum tendineum, the gastro-hepatic and gastro-splenic omentum and in the vast lymphatic beds in the peritoneum around the pylorus. Also we found them in the lymphatics of the ligamentum latum.

With ligated lymphatics while alive, the peritoneum, in 30 minutes, absorbed (95 c. c. m.) 3.63 per cent. of the body weight.

With ligated lymphatics while dead, the peritoneum, in 30 minutes, absorbed (55 c. c. m.) 2.1 per cent. of the body weight.

The first blue color in the urine appeared in 30 minutes.

Experiment No. 21. Dog, male. Weight, 9 pounds. Chloroformed and left innominate vein ligated. 100 c. c. m. of a solution of 98 c. c. m. of  $H_2O$  plus 2 c. c. m. of a 5 per cent. solution of potassium ferrocyanide, plus sufficient Na.Cl. to make a 1 per cent. Na.Cl. solution, was injected at 2:25. Ten minutes later, at 2:35, 100 c. c. m. more of the same solution was injected. Five minutes later, i. e., 15 minutes after the first injection, the dog suddenly died from the chloroform. No reaction could be gained from the urine by the addition of the ferric salt, though tested every five minutes. By testing the pleural cavity no reaction of the blue color appeared. In 15 minutes 100 c. c. m. were absorbed.

Experiment No. 22. The same dog, dead. The dog's peritoneum absorbed 30 c. c. m. of the same kind of fluid in 15 minutes. No oedema or peritoneal extravasation could be observed. Berlin blue was added to the solution injected into the dead animal.

Microscopically.—The diaphragm and gastro-splenic omentum did not show abundant filling of the lymphatics, but a spare quantity was found in the diaphragmatic and gastro-splenic omentum.

With ligated lymphatics while alive, the peritoneum, in 15 minutes, absorbed (100 c. c. m.) 3. per cent. of the body weight.

With ligated lymphatics while dead, the peritoneum, in 15 minutes, absorbed (30 c. c. m.) 0.9 per cent. of the body weight.

No blue reaction in the urine.

Experiment No. 23. The centrum tendineum of the diaphragm of a man aged 28. Dead about 30 hours from violent and universal interstitial and parenchymatous nephritis, was placed in a watery solution of Berlin blue for about 5 hours. It was then stained with  $\frac{1}{2}$  per cent.



Ag. NO<sub>3</sub> solution for 15 minutes. This specimen showed most typically absorption of Berlin blue particles. The blue granules were located in the lymphatic vessels, but especially the large valved trunks as they radiated toward the zona peritendinea.

Experiment No. 24. Diaphragm of girl 13 years old. Dead 28 hours. Was placed in a watery solution of Berlin blue for several hours and showed after staining with Ag. NO<sub>3</sub> absorption of the blue granules.

Microscopically.—This specimen showed beautifully the perforation of the membrana limitans of the centrum tendineum.

Experiment No. 25. Rabbit. Female. Weighed 1,450 grammes. Left innominate and right jugular veins ligated. Injected 50 c. c. m. (H<sub>2</sub>O., 48 c. c. m. plus 2 c. c. m. of potassium ferrocyanide of 5 per cent. plus a 1 per cent. Na. Cl. solution) into the abdomen. The reaction of urine was tested every 5 minutes. No urine reaction occurred until 30 minutes after the injection, and then only a light tint of blue. Rabbit killed with chloroform and collected 20 c. c. m.—absorbed 30 c. c. m. in 30 minutes. The rabbit was allowed to wake up after the injection into the peritoneal cavity.

With ligated lymphatics while alive the peritoneum, in 30 minutes, absorbed (30 c. c. m.) 2.06 per cent. of the body weight.

The blue reaction occurred in the urine in 30 minutes.

Experiment No. 26. Same rabbit (dead). Injected 50 c. c. m. of H<sub>2</sub>O. a 1 per cent. solution of Na. Cl. and sufficient Berlin blue to color the fluid. In 30 minutes 14 c. c. m. were collected, loss 2 c. c. m. Absorbed 33 c. c. m. The proximal side of the right jugular vein was very oedematous for one inch, and a similar condition existed about the proximal side of the ligature in the innominate vein. The urine was pressed out of the vagina by pressing on the bladder. No peritoneal oedema could be seen.

With ligated lymphatics while dead the peritoneum absorbed in 30 minutes (34 c. c. m.) 2.34 per cent. of the body weight.

Microscopically.—The colored granules are found (a) widely distributed into the lymphatics of the diaphragm; (b) the lymphatics of the gastro-splenic omentum; (c) in the lymphatics of the broad ligaments.

This specimen was a good sample to prove that Schweigger-Seidel was in error when he stated that washing the peritoneal membrane would prevent the interendothelial lines from arising on the application of Ag. NO<sub>3</sub>. This specimen was placed in a bottle of distilled water and put in my pocket and carried a mile on a bicycle, when it was found that though Ag. NO<sub>3</sub> called up the interendothelial lines, yet some were very faint.

Experiment No. 27. Dog. Male. Weighed 8 pounds. Anesthetized

with chloroform. Right and left innominate veins ligated. Duration of the experiment, 30 minutes.

2:03—100 c. c. m. 2 per cent. Na.Cl. solution and 2 c. c. m. 5 per cent. potassium ferrocyanide.

2:22—No blue in urine.

2:28—Slight tinge in urine.

2:33—Decided blue reaction. Dog killed with chloroform. Recovered from dog's peritoneal cavity 30 c. c. m. in excess of injection (130 c. c. m.) 30 c. c. m. was added to the injected fluid. Nothing abnormal observed. The 2 per cent. Na.Cl. solution was hypertonic and followed the law of osmosis.

With ligated lymphatics while alive, the peritoneum, in 30 minutes, absorbed (25 c. c. m.) 1 per cent. of the body weight.

Experiment No. 28. Same dog, dead. 2:43—100 c. c. m injected (of Berlin blue and  $H_2O$ ). 3:13—Recovered 75 c. c. m. Absorbed 25 c. c. m. No blue reaction in chest cavity one hour after first injection. The urine was pressed out of the bladder and tested with ferric chloride. As the dog had a full stomach and intestines one would consider the intra-abdominal pressure even higher than a normal dog. But the Na. Cl. solution was 2 per cent., i. e., hyperistonic.

With ligated lymphatics while dead, the peritoneum, in 30 minutes, absorbed (25 c. c. m.) 0.83 per cent. of the body weight.

Microscopically.—This dog showed nothing abnormal except the small amount of Berlin blue found in the centrum tendineum. But the particles of Berlin blue were distinctly deposited in the omentum about the pylorus where a rich network of lymphatics exists.

Experiment No. 29. Rabbit, female. Weight, 713 grammes. Both innominate veins ligated. Injected into the abdominal cavity 100 c. c. m. of (2 per cent. Na.Cl. plus 2 c. c. m. of a 5 per cent. solution of potassium ferrocyanide plus  $H_2O$ ). Rabbit allowed to wake up partially. A blue color appeared in the urine 25 minutes after the peritoneal injection. Killed in 30 minutes after the injection. Recovered 15 c. c. m. lost 10 c. c. m. Absorbed 75 c. c. m.

With ligated lymphatics while alive, the peritoneum, in 30 minutes, absorbed (75 c. c. m.) 10 per cent. of the body weight.

With ligated lymphatics the blue reaction appeared in the urine in 25 minutes.

Experiment No. 30. The same rabbit, dead, was injected with 100 c. c. m. of same solution except the ferrocyanide. A little Berlin blue was added to mark the lymphatics. In 30 minutes the dead rabbit's peritoneum absorbed 57 c. c. m, 43 c. c. m. were recovered. The standard of absorption of the same animal, dead or living, is rela-

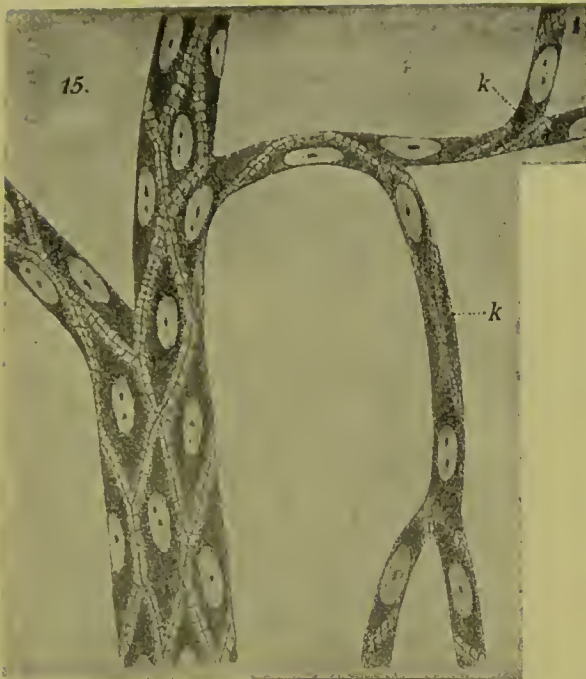


FIG. 220.—(A. Kolossow, 1895.) Endothelia of a small artery and capillary of the pia mater of a two-months-old dog, semi-diagrammatic, showing the oval nuclei, and the anastomotic processes in the interendothelial space.

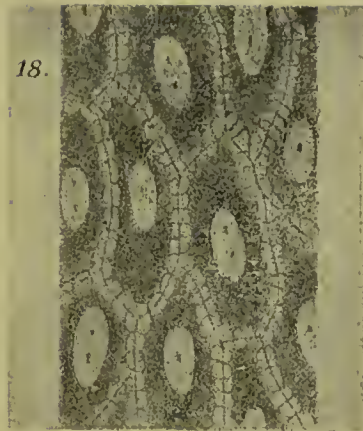


FIG. 221.—(A. Kolossow, 1895.) Endothelia of the ductus thoracicus of a large dog, showing no cilia on the cover plates, but distinct oval nuclei with nucleoli and excellent examples of anastomotic protoplasmic processes binding cells into colonies.

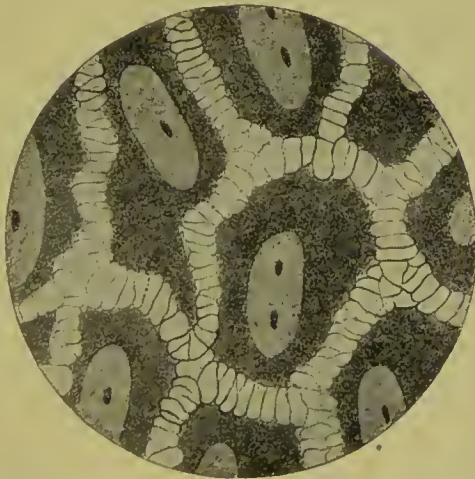


FIG. 222.—(A. Kolossow, 1895.) Endothelia of the peritoneum on the stomach of the axolotl, treated with osmic acid and tannin. The microscope is so adjusted as to see as deep into the interendothelial space as possible. One may observe that the protoplasmic anastomotic processes which bind the cells into colonies and which lie in the interendothelial space are sinuous. Some appear like rings. The superficial anastomotic processes are thinner and finer while the deep ones are thicker and stronger. Observe the nucleoli in the well oval nucleus.





tively the same. With ligated lymphatics while dead, absorbed (57 c. c. m.) .8 per cent. of the body weight.

In the recorded experiments, the highest per cent. of absorption of the peritoneum during life was 10 per cent. of the body weight. The lowest per cent. of absorption was .88 per cent. The average absorption was 4 per cent. of the body weight.

While dead the highest per cent. of absorption was 5.5 per cent. of the body weight. The lowest was .83 per cent. The average absorption was 2.32 per cent. of the body weight. The 10 per cent. body weight absorption in thirty minutes while alive. The 5.5 per cent. of body weight absorption of fluids by the peritoneum while dead occurred in 40 minutes. Should the time of absorption be protracted, doubtless the quantity of fluids absorbed by the peritoneum would exceed by far 10 per cent. of body weight.

Experiment No. 31. Rabbit, male; weighed about 700 grammes. Injected 100 c. c. m. of a solution composed of (2 c. c. m. of a 5 per cent. K. ferrocyanide plus 98 H<sub>2</sub> O. plus 3 per cent. Na. Cl.) at blood heat at 3:27; 3:37 tinge of blue in urine; 3:42 quite blue; 3:47 deep blue (his bladder was very full from the beginning); 3:57 killed and collected 45 c. c. m., wasted 5 c. c. m., absorbed 50 c. c. m. in 30 minutes. At 4:02 injected 100 c. c. m. of 3 per cent. Na. Cl. solution. Collected 50 c. c. m., wasted 5 c. c. m., absorbed 45 c. c. m. in 30 minutes. Sufficient Berlin blue was added to the first injection to trace out the lymphatics.

Microscopically.—The diaphragm showed distinct intertendinous lymph deposits of blue. The sub-endothelial lymphatics were well filled with blue. The Berlin blue was well and extensively deposited in the lymphatics of the diaphragm.

While alive with open lymphatics the rabbit's peritoneum in thirty minutes absorbed of a 3 per cent. Na. Cl. solution (50 c. c. m.) 6.2 per cent. of the body weight. While dead the rabbit's peritoneum in thirty minutes absorbed of a 3 per cent. solution (45 c. c. m.) 5.6 per cent. of the body weight.

With open lymphatics the urine became blue in ten minutes after the peritoneal injection. Though this fluid was 3 per cent. Na. Cl. solution (hypertonic), it was quite rapidly absorbed. Doubtless it became isotonic and then hypertonic whence absorption occurred. The centrum tendineum showed very typically the well filled lymphatics with Berlin blue which demonstrates their connection with the lymphatics of the intertendinous spaces. The lymphatics of the centrum tendineum show well their varied shape due to valves and also to the compression which some are subject to as they pass through the narrow space between two tendons. This is plainly seen when a large, well-filled lymph vessel suddenly and sharply bends and narrows as it passes through

the narrow space between two bundles of tendons. In thirty minutes while alive and thirty minutes while dead the vast lymphatics of the diaphragm were filled to a wonderful degree of distension and extension. The leucocytes were emerging from the sub-endothelial tissue through the interendothelial space to attack the particles of Berlin blue (a) to digest them; (b) to sterilize them and (c) to imprison them by burying them in exudates.

Experiment No. 32. Rat. Small size. Injected into abdomen a watery solution of Berlin blue at  $2\frac{1}{2}$  to 3 oz. Killed in thirty minutes. Almost every drop was absorbed (non-anesthetized). No oedema. The large omentum majus of the rat was not entangled by the Berlin blue granules. Scarcely any colored granules were found in the peritoneal cavity. The rat had six foetuses in one horn of the uterus and three in the other about the size of small peas.

Staining with gold chloride and acetic acid brought out the peritoneal nerves. Ag.  $\text{NO}_3$  demonstrated endothelia and very typical stomata vera on the gastro-splenic omentum and centrum tendineum. The blood vessels and lymph vessels showed up excellently. The colored granules of Berlin blue are very much scattered. They are sparingly deposited in some lymph vessels lying adjacent to large blood vessels. Some few are found in other lymph channels of the diaphragm. In general the fine granules of Berlin blue were deposited in the lymphatics accompanying the blood vessels. In the rat the invagination of the blood vessels by lymphatics is quite evident. The rapid circulation of the rat had doubtless swept the chief quantity of the fine colored granules beyond the diaphragmatic lymphatics in thirty minutes.

The interendothelial space is very plain in the rat, with its parallel double lines and transverse anastomotic protoplasmic processes. The stomata vera are typical. The leucocytes can be observed in various positions below the endothelia attempting to come to the surface.

The gastro-splenic omentum presents a very variegated appearance of germinating cells. Beautiful growths of lymphatic vessels appear in stages of vacuolation.

#### OBSERVATIONS ON ABSORPTION OF PERITONEAL FLUIDS FROM AUTHOR'S EXPERIMENTS.

The fact to account for is, that fluids disappear from the peritoneal cavity of living and recently dead animals. To give a satisfactory account of the problem the paths of absorption from the peritoneum must be studied. Three general questions may be asked.

1. Does the peritoneal fluid enter the blood directly through the walls of the blood vessel?

2. Does the peritoneal fluid enter the blood by way of the lymphatics?



## 3. By what force is peritoneal absorption accomplished?

We will consider the last question under the following heads:

## 1. Is peritoneal absorption due to "vital" forces of the endothelial cells?



FIG. 223.—(Author.) Drawn from a rabbit's omentum. The rabbit was killed by ether, the abdomen carefully opened and in situ the omentum brushed with a little cotton on a tooth pick. The brush should be rubbed three or five times over the part and wet in distilled water, better in rabbit's own peritoneal serum. The figure is drawn under oc. 4, obj. 8a, (Reichert.) The silver nitrate used was one-half per cent., remaining on the parts some four minutes and then mounted in glycerine. The figure shows well the stomata vera CS. It will be observed that at CS the stomata are closed while toward the middle several remain open. Notice the stomata vera CS are lined with granular polyhedral cells in which one can occasionally see a nucleus. These little mouths are found in all kinds of states as regards being open or shut. These are the vertical lymphatic canals which preserve a direct communication between the peritoneal cavity and the sub-peritoneal lymph channels. Besides they are a source of new endothelia to supply the place of degenerating comrades. N points to nucleus of the endothelial cell which is clearly brought out by logwood applied three minutes. SS points to black dots or open points. These are known as stomata spuria and Virchow calls them lymph corpuscles; Recklinghausen and Oedmansson name them connective tissue corpuscles jutting upward between the junction of two or more opposing endothelial cells. SV represents stomata vera open. I did not sketch in this figure other apertures which I have styled intra-endothelial stomata. The figure is drawn as nearly natural as possible.



FIG. 224.—(Author.) A. New-born human omentum showing stomata vera and spuria and very irregular shape and size of endothelia. 1, stomata vera, a typical case; 2, 3, 4, other stomata vera; 5, stomata spuria; 6, stomata verum with its granular polyhedral cells; 7, 8, endothelia. B. Drawn from a foetal (two months) pig's diaphragm, abdominal side. It shows a typical stomata verum (1) and it represents it distinctly as shimmering through the common endothelia (2 and 3). There is a round black dot in the center of the stomata vera through which growth process takes place. The endothelia on this foetal pig's diaphragm are much more regular in shape and size than on new-born humans.



FIG. 225.—(Author.) From a young dog's kidney. The peritoneum was snipped off with a pair of scissors. (Oc. 2, obj. 8a, R.) Ag. NO<sub>3</sub>. Note the grouping of cells. 1 and 2 are two endothelial cells around which eight cells are grouped. The cells are quite uniform. The fibrous and elastic network below the endothelia is very dense. Two endothelial cells are not drawn brown from the Ag. NO<sub>3</sub>.

2. Is it due to stomata?
3. Is it caused by increased intra-abdominal pressure?
4. Is it produced by filtration?
5. Is it owing to capillary or molecular imbibition?
6. Does peritoneal absorption rest on the laws of osmosis?
7. Is peritoneal absorption so-called "vital" process of the endothelial cells?

The diaphragmatic peritoneum will absorb particles of Berlin blue suspended in fluid 72 hours after death, as proved by our experiments. Immediately, or shortly after death, from the inspection of the preceding experiments, the peritoneum will absorb about one-half as much as the same animal living. It may be claimed that the peritoneum is not dead, has not lost its vital activity, with merely cessation of the cardiac pulsation. Doubtless it will preserve vital or living properties for, say, a half a dozen hours, but few would claim life or living properties for it beyond 24 hours. The fact that the peritoneum in recently dead animals will absorb was made known by Daniel Von Recklinghausen, cultivated by Ludwig and his celebrated pupils and recently revived in a striking way by Prof. Hamberger of Utrecht, Holland, to whose labors I am indebted. Since, in spite of energetic trauma or thermic injury to the endothelial cells the absorption proceeds, it must exclude so-called "vital forces." As a general deduction from experiments we think that so-called "vital forces" should be excluded from the absorption of peritoneal fluids. It belongs much more to the field of physical processes. H. J. Hamberger injected solutions of Na.Fl. into the peritoneal cavity from 0.1 per cent. to 0.4 per cent., which last dose proved fatal in fifteen minutes, yet peritoneal absorption continued in spite of chemical injury to the endothelial cells. He injected 150 c. c. m. of a 2 Na.Cl. solution which contained also a 0.4 per cent. solution of Na.Fl. In fifteen minutes the rabbit was dead; 100 c. c. m. of fluid was found in the peritoneal cavity; absorbed 47 c. c. m. even under the injurious influence of the Na.Fl. Autopsic inspection revealed hemorrhage condition of the peritoneum. Also the use of a 3 per cent. H. Cl. solution plus Na.Cl. 2 per cent. solution in dog's peritoneum did not injure the peritoneum sufficient to stop absorption. Peritoneal absorption, as Hamberger shows, occurs in dogs quite rapidly under a temperature of 92 degrees centigrade. Peritoneal absorption proceeds under thermic, chemical or physical injuries after death. It is doubtless not a vital process, but a physical process.

2. Is peritoneal absorption of fluid due to stomata?

After two years of continuous microscopic investigation on various animals I am convinced that the peritoneum possesses stomata. The stomata play some role in absorption as they are not useless structures. The stomata are not equally distributed over the whole peritoneum, but

present themselves typically on the diaphragmatic peritoneum. From the time (1873) Bizzozero discovered that the *membrana limitans* was perforated in the region of the diaphragm an apparently physical base has been given to the explanation of the absorption of solid particles which were suspended in the peritoneal fluids, especially in the region of the diaphragm. The *stomata vera*, that is, those stomata located at the common junction of several endothelial plates, appear to possess a splinter-like action. They open and close or contract and dilate, and thus regulate, or aid in regulating, peritoneal currents. Particles of Berlin blue were found in our experiments deposited in several places outside of the diaphragmatic peritoneum in localities where no *membrana perforations* could be found and, hence, we think the perforations in this basement membrane cannot account for all the wonderful power of the diaphragm to absorb particles of solid matter. It is not, so far, definitely decided whether the diaphragmatic peritoneum is much superior to other localities of the peritoneum to absorb fluid, but experiments point in that direction, for colored fluids pass from the peritoneal cavity and color the thoracic glands. It is definitely settled from experiments that the diaphragmatic serosa is vastly superior to other peritoneal locations to absorb solid particles of matter; in fact, it is its chief locality.

In the matter of peritoneal absorption we place the stomata as definite factors. Schweigger-Seidel claims that there are definite perforations in the *membrana limitans* covering the *cisterna lymphatica magna*, and no histologists deny that stomata exist on the peritoneal serosa of this lymph sac. Is the diaphragm, especially the *centrum tendineum*, not a remnant of these amphibian lymph sacs? Since the stomata are debatable grounds in the peritoneum, we will omit further discussion of the matter. We leave it, however, with the belief that the stomata acts an important part in the absorption of peritoneal fluids.

In open canals, as are considered to exist on the diaphragm, it does not make so much difference in regard to the concentration of fluids; for if the subperitoneal lymph vessels stand in open communication with the peritoneum, the laws of osmosis will not assume a control so prominent in general peritoneal absorption. It is, however, difficult for the general investigator to overlook the recorded experiments of Von Recklinghausen and others where he announces that he observed the milk globules pass into the stomata.

The third question as to peritoneal absorption being due to intra-abdominal pressure we must consider as purely mechanical. I mean by intra-abdominal pressure muscular tension, plus atmospheric pressure, both of which act as a purely physical or mechanical process. To consider that peritoneal fluid passes out of the peritoneum by intra-abdom-



inal pressure is to assume at once that the pressure in the abdomen is higher than it is in the immediately adjacent tissue. Our data as to facts in regard to the variation of the pressure in different tissue are still too meagre to make any strong assertions. Again, so far as I understand the word filtration it must be included in the term intra-abdominal pressure. Filtration is a process which demands physical force. It means that force is greater on one side of a definite line than on another. To have filtration of peritoneal fluids we must admit the pressure in the abdominal cavity to be higher than the adjacent tissue. Filtration, then, is a mechanical process or that kind of absorption

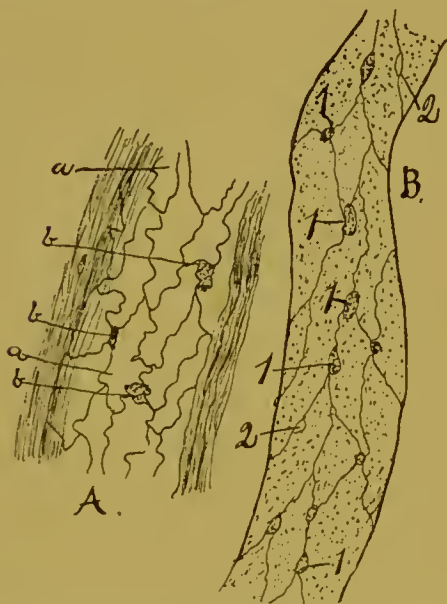


FIG. 226.—(Frey, 1875) A. Lymphatic canal of the larger intestine of guinea pig to show stomata vera. a, Endothelia of the vessel; b, stomata vera.

FIG. 227.—(Author.) B. Capillary vessel of mesentery to illustrate stomata vera. 1, 1, Stomata vera; 2, 2, Stomata spuria in the interendothelial space of the vessel wall.



FIG. 228.—(Thoma, 1896.) Capillary from the mesentery of the dog after injection with dilute Ag. NO<sub>3</sub> and staining with borax carmine X260.

accomplished by intra-abdominal pressure. Hence, though the terms intra-abdominal pressure and filtration may assume and possess variations of meanings, yet we think it practical to include both under the term mechanical pressure. In the living animal the muscular tension is continually varying, as is observed in respiratory movements or the movements of single muscles or groups of muscles, all of which increase mechanical pressure. After death, or even before death, mechanical (intra-abdominal) pressure is increased by the development of gases. We know from experiments that massage of the abdomen increases peritoneal absorption, as also does walking or movement of the animal. Hanging the animal by the heels also enhances peritoneal absorption.

All these physical forces mean increase of intra-abdominal pressure, and hence increase of peritoneal absorption.

So far we are unable to estimate in any way the exact role played by the increased peritoneal absorption by the mechanical pressure. When the animal is dead mechanical pressure in peritoneal absorption must play a significant role, while osmosis, one would think, played a less significant role.

Intra-abdominal pressure, filtration or mechanical pressure certainly assumes a significant part in the absorption of peritoneal fluids. Wegner asserts that a greater quantity of peritoneal fluid is absorbed in the same time when the peritoneal cavity is more distended with fluid than when it contains only a small quantity. As a sample he gives: a rabbit's peritoneum was injected with 200 c.c.m., and in one hour 134 c.c.m. was absorbed by the peritoneum. Again, a rabbit was injected with 100 c.c.m. and only 50 to 60 c.c.m. were absorbed. However, it may be claimed that if more fluid be injected into the peritoneal cavity it will necessarily come in contact with more peritoneal surface. The clinician knows well that by pressure bandages he can hasten the absorption of peritoneal fluids. Intra-abdominal pressure up to a certain point will hasten absorption until a certain tension is passed whenever the peritoneal absorption rapidly diminishes. This may be noted in ascites. If a patient has a large abdomen full of ascitic fluid it will often remain so for a long period. But if we lessen the hydrostatic pressure by drawing off a small quantity of the ascitic fluid the remainder is easily and rapidly absorbed. The absorption of the ascitic fluid was started by taking off the excessive intra-abdominal pressure. Hamberger demonstrated by experiments that in increasing intra-abdominal pressure at first the arterial pressure increases, but by further increase in the intra-abdominal pressure the blood pressure decreases. There is a relation then between intra-abdominal pressure and blood pressure. The sensitive heart tries to overcome increasing intra-peritoneal pressure and bring about a physiologic balance by inducing a more rapid blood current through the peritoneal region so that absorbed particles of fluid may be rapidly swept on in a current, allowing room for more fluid to be absorbed and swept on. The fluid in a distended ascitic peritoneal cavity when it becomes real high in pressure will compress the veins which are the chief outlet and the absorption comes to a standstill. Intra-abdominal (mechanical) pressure works in two distinct ways, viz.: One factor presses the peritoneal fluid more vigorously to the walls of the interstitial spaces and blood vessels and hence hastens the peritoneal absorption. While the second factor compresses the blood vessels or interstitial spaces which lead the fluid away, in other words, narrows the lumen of the drains in general and thus checks peritoneal absorp-

tion. The excessive intra-abdominal pressure affects the soft and compressible veins or vessels chiefly and not the stiff, thick-walled arteries. As lymph is generally looked on as a filtrate under pressure of the blood plasma, so when the peritoneal cavity contains abnormal quantities of fluid its expression from the peritoneum may be looked on as filtration under the intra-abdominal (mechanical) pressure, or a product of the physical product of filtration. It is a curious feature that when a disturbed balance occurs between the appearance and disappearance of lymph in the peritoneal cavity, the favor is toward the excess of lymph, i. e., there is more filtered into the peritoneum than is filtered out of it. As an example of support of the filtration hypothesis, Starling reports that ligation of the portal vein affects the arterial pressure little, but the pressure rise in the vein behind the ligature is enormous. In consequence of this there is a large rise of pressure in the capillaries of the intestines and spleen so that the spleen swells and the intestines become black from venous congestion and hemorrhage occurs in the intestinal mucosa. The significant report of this experiment is that the lymph flow from the thoracic duct is increased four or five times.

This shows that the passage of lymph from one place to another is favored by mechanical pressure of filtration. Starling also reports that ligation of the vena cava just above the diaphragm increases the lymph flow ten to twenty fold. The majority of experiments appear to cast their weight in favor of increased capillary tension as the cause of increased lymph flow. This is highly in favor of the filtration hypothesis. The balance between absorption and secretion in the peritoneal cavity is very delicate and when any excess of fluid is injected into the peritoneal cavity it at once makes the pressure in that cavity distinctly higher than the pressure in the adjacent tissue. The result must be filtration in the direction of least resistance to lymph. Further information of lymph flow and its relation to filtration may be found in the excellent article by W. S. Lazarus-Barlow in the *Journal of Physiology*. Intra-abdominal and vascular injection of fluid raises the capillary pressure and an increase flow of lymph occurs in the peritoneum. This is filtration from pressure. It may be proved that it is filtration from capillary pressure, for if we bleed an animal 300 c. c. m. and then inject 300 c. c. m. of fluid in the vessels, the excessive lymph secretion fails. Hence, filtration of fluids increases under increased capillary pressure. Of course, the amount and composition of peritoneal absorption depends much on the pressure, nature and condition of the membrane, i. e., the peritoneum. The higher the capillary pressure or the peritoneal pressure, the more easy does filtration occur, because it makes the capillary and peritoneal membranes more permeable by stretching or extension. Slight extension of peritoneum or especially capillary membrane doubtless produces



interruption in the interendothelial spaces which close by contraction as soon as the distension is removed. Filtration has relation to two distinct factors, viz.:

1. The permeability of peritoneal or capillary membranes; and, 2, on the mechanical pressure.

As a resume of filtration and intra-abdominal (mechanical) pressure in regard to the absorption of peritoneal fluids, we may say:

1. The absorption of peritoneal fluids is hastened by mechanical pressure.

2. The hastening of the absorption of peritoneal fluids by mechanical pressure is considerable. Hamberger has an experiment to demonstrate this proposition. In a rabbit with a ligated thoracic duct with a pressure of 9 c. c. m., there was absorbed 35 c. c. m. of a 0.9 per cent. Na.Cl. solution (isotonic with the blood serum) while with a pressure of 14 c. c. m. there were absorbed 72 c. c. m.

3. If the intra-abdominal pressure exceeds a certain border the absorption of peritoneal fluids decreases.

4. Since increased intra-abdominal (mechanical) pressure or filtration hastens remarkably peritoneal absorption, it points to a physical process and not a "vital" force.

5. The blood stream is influenced by intra-abdominal pressure, as may be observed in the tympanitis when the distended bowel very materially lessens the lumen of the blood vessel by elongating it. Then the heart attempts to respond by a more powerful beat. Hence in a moderate increase of intra-abdominal pressure the heart attempts to overcome the obstruction by a more powerful beat, and hence the rise of blood pressure. But if the intra-abdominal pressure exceeds certain border the heart is not in a condition to keep up a heavy beat, as it begins to receive less blood and the arterial pressure sinks. This is shown by Hamberger's experiment just noted.

The fact that the lymph from the peritoneum contains less proteid than the blood plasma does not militate against filtration as asserted by Starling's hypothesis. If serum be filtered through an ordinary porous filter paper the filtrate will remain about the same as the original, but if filtered through a finely meshed paper or porous clay cell or an animal membrane it is found that the filtrate is poorer in proteid than the original. The larger proteid molecules are apparently unable to pass through the fine mesh-work of the fine filter. That lymph, natural peritoneal fluid, has been viewed as a filtrate, expressed into the peritoneum by intra-capillary pressure, has been the standard of thought for almost 100 years. It may be noted in the philosophic works of Dr. Hewson, in whose premature death medical science bore a loss. It appears in Dr. Hale's works, but to Dr. Karl Ludwig and his industrious

pupils we owe the systematic advocacy of the filtrate hypothesis. According to the filtrate hypothesis, the amount of lymph in any given locality is proportionate to the difference of pressure between the capillaries and the intra-vascular space. Ludwig found that ligating the plexus pampiniformis increased the amount of lymph in the testicle from filtration or mechanical pressure. At any rate, it increased intra-capillary pressure and produced increased amount of lymph. Under these conditions increased interstitial (peritoneal) pressure would produce a flow of lymph in its interstitial membranes by filtration. Hence, in filtration, we must observe the conditions of the membranes whether they be permeable to large (proteid) or small ( $H_2O$ ) molecules. In other words, the nature of the membrane and the nature or size of the molecule must be considered.

The conditions of increase and excess of intra-abdominal (mechanical) pressure in the absorption of peritoneal fluid is noticed in clinical labors by the fact that in excess of mechanical pressure in peritoneal, ascitic fluid, the fluid remains stationary, but if one will bring the mechanical pressure of the ascitic fluid to a lower point by tapping, the remaining ascitic fluid is often rapidly absorbed. As the clinician remarks, the pressure is taken off and the fluid absorbs. When ascitic fluid widely distends the abdomen, mechanical pressure is exercised so high that the lumen of veins and capillaries which carry the blood through the abdomen and away from it are narrowed. The result is distinct oedema.

The sixth question is peritoneal absorption owing to capillary or molecular imbibition. A. Fick separates imbibition into capillary and molecular. We have all observed capillary attraction in the oil-wick, and it is easy to see the elevation of fluids higher in a capillary tube than outside of it. Capillary imbibition is the absorption of fluid in the pores or capillaries of porous substances or masses. Molecular imbibition is the passing of fluids into homogeneous masses. It is a form of cohesion of matter, an attraction of fluid particles toward solid particles. Under the term imbibition we understand that any tissue will take up more fluid than it ordinarily contains. But as to the amount of fluid any tissue will take up, the process of imbibition is always a limited one and the amount of absorption of peritoneal fluids must also be a limited one.

Imbibition is well seen in the dorsal aspect of the peritoneum in dead animals when fluid has been injected and allowed to remain there. I saw it but very little in living animals, especially in short experiments. If fluid be injected into the abdominal cavity it can be absorbed by imbibition; the sub-endothelial tissue through capillary imbibition can conduct it into the vessels which finally carry it into the general circu-

lation. All the absorption in the peritoneum is aided by the lymph or blood vessels, immediately carrying away all particles absorbed so that more room is made for new ones. In this way imbibition may continue for considerable time, repeating its absorption of limited volumes of fluid which are in turn swept on by the currents in the vessels. The absorption of repeated volumes of fluid by imbibition is most typical in the living when both blood and lymph streams sweep them on into the general circulation. But in the dead animal the blood stream fails and the lymph stream continues only for a limited time, so that in the dead animal the absorption of peritoneal fluids by imbibition is limited to primary volumes.

As to the sixth question, "Does peritoneal absorption rest on osmotic pressure?" we are at once plunged into difficulties on account of the complication accompanying the process of osmosis.

Osmosis, we must assume, plays a very important role in the absorption of peritoneal fluids. The most significant fact in regard to the absorption of peritoneal fluids in confirming its relation to osmosis is, that peritoneal fluids disappear in inverse ratio to their osmotic pressure. The less concentrated a solution of Na.Cl. is, injected into the peritoneum, the more rapid it becomes absorbed from the peritoneum. In osmotic pressure we have a tangible rule to follow, but life's processes, often so inscrutable, involve so many conditions that the rules so far established are not universally agreed upon.

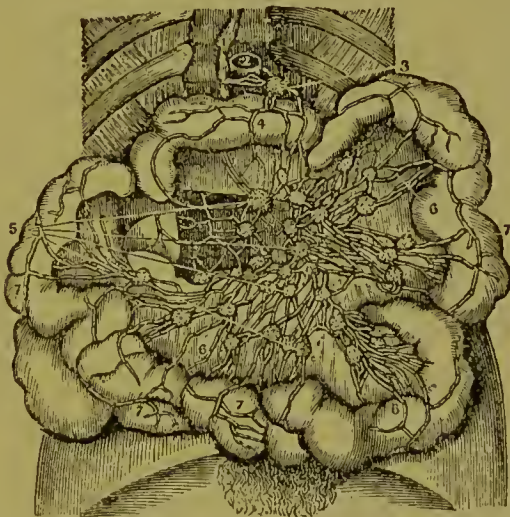


FIG. 229.—(Horner, 1851.) A view of the lymphatics of the small intestines, dead from ascites. 1, thoracic duct; 2, section of the aorta; 3, 3, glands around the aorta, which receive the lymph from the peritoneum and intestines and give off vessels to the thoracic duct; 4, superficial lymphatics of the intestines; 6, 7, lymphatics of the intestines and mesentery.

What is osmotic pressure or osmosis? Osmosis is the tendency of fluids to mix. If two fluids of different density be placed in a vessel on each side of a membrane the thinner fluid will quickly begin to flow toward the thicker. The thicker fluid, i. e., the one having a higher density, has a greater osmotic pressure than the thinner. Osmose means that fluids of different density will form currents toward each other. The standard of osmotic pressure is the blood of the animal on which the experiment is performed. Fluids having an osmotic pressure equal



to the blood may be called isotonic. If this standard be measured by Na.Cl. solution, authors claim that a solution of Na.Cl. varying from 0.73 per cent. to 0.92 per cent. is isotonic with the blood. Na.Cl. solutions of 0.73 per cent. to 0.92 per cent. injected into the peritoneal cavity are absorbed very slowly or not at all, because they are isotonic or about equal osmotic pressure with the blood. If such isotonic solutions do absorb, they likely first become hypotonic.

We also have what might be called hypotonic solutions, that is, solutions which have an osmotic pressure below a Na.Cl. solution of 0.73 per cent. Such hypotonic solutions absorb from the peritoneum rapidly, and the more rapid the less concentrated or the more hypotonic they are.

Finally, we have solutions which may be termed hypertonic. They have an osmotic pressure above a 0.92 per cent. Na.Cl. solution. Hypertonic solutions do not become absorbed, or but slightly absorbed when injected into the peritoneal cavity. They become less absorbed, the more concentrated they are, or, in other words, the more hypotonic they are. If hypertonic solutions become absorbed they most likely become first isotonic and then hypotonic. For example, the fluid injected in experiment No. 26, a dog, as 2 per cent. Na.Cl. solution which was hypertonic to the blood and we collected 30 c. c. m. in excess of what we injected in thirty minutes. The 2 per cent. Na.Cl. solution injected into the peritoneum was more dense, or had a greater osmotic pressure than the body surrounding the peritoneum, hence, by the law of osmosis the adjacent thinner fluid tends to flow toward the thicker and more dense fluid in the peritoneum. We will here quote Heidenhain's views of osmosis, condensed into four propositions.

1. "If two watery solutions of the same osmotic pressure are separated by a membrane through which diffusion can take place, no change in volume occurs on either side of the membrane."

2. "If the solutions on either side of the membrane are of unequal osmotic pressure, water passes from the side where the pressure is less to the side where the osmotic pressure is greater."

3. "The osmotic pressure of a solution is equal to the sum of the partial pressures of the various dissolved substances."

4. "If the solutions on the two sides of the membrane have the same total osmotic pressure, but unequal partial pressures of their various constituents, each constituent of the solution passes from the side where it has the higher partial pressure to the other side. No change in the volumes of water on the two sides takes place."

No one doubts, who has experimented, the significant role of osmosis in peritoneal absorption. However, all of Prof. Heidenhain's proposi-

tions will not be generally accepted. The permeability of different membranes to different substances is not exactly known.

Starling goes so far as to deny all of Prof. Heidenhain's propositions, except the one marked No. 3. The propositions 1, 2 and 4 require conditions seldom met in the body. In osmosis the nature of the membrane must be carefully considered. The initial and final rate of osmosis demands attention.

J. B. Leathes has shown that five minutes after the injection of sugar and salt into the blood vessels the osmotic pressure of the blood and lymph (peritoneal fluid) have become equal. It has been found that solids in the solutions act like gases. There can be no difference in pressure between two gases in a vessel which have no partition between them or even divided by a freely permeable serosa. We can assert that similarly there can be no difference between the osmotic pressure of fluids separated only by a freely permeable membrane. At first a slight difference may arise, but final osmotic pressure must be equal. If we take as an example experiment No. 31, where we injected into the peritoneal cavity 100 c. c. m. of a 3 per cent. Na.Cl. solution. This was hypertonic to the blood and would in all probability attract fluid to it from the blood until its osmotic pressure was equal to the blood, but mechanical (filtrating) pressure was greater than osmosis and, hence, the fluid rapidly disappeared from the peritoneal cavity, but not necessarily into the blood, more likely into the interstitial spaces. Doubtless, the equalizing of the osmotic pressure of the peritoneal fluid began at the edges, or that portion of the fluid lying in contact with the peritoneum. E. H. Starling, of London, has shown that proteids do not pass through a membrane, as the wall of the capillaries, readily. This is an important fact, for, in consequence, the fluids transmitted from the capillaries are poor in proteids while a large amount of proteids are left in the lumen of the capillaries, which, of course, must exercise an osmotic pressure on fluid outside the capillaries. For example, if blood serum be placed in a funnel over whose large end is stretched a peritoneal membrane soaked in gelatine, and the funnel be inverted and immersed into salt solution (isotonic or hypotonic with the serum), within 48 hours the salt solution will pass into the funnel and raise the fluid in the stem to a considerable height (Starling). This shows that the non-diffusible portion of blood possesses considerable osmotic pressure. The osmotic pressure of albumen is small.

Osmosis is the law which holds the physiologic balance between the intra- and extra-vascular fluids. As remarked by Starling, the osmotic pressure of the blood serum will be in proportion to the force expended in the production of this extra-vascular fluid, so that any time there must be a balance between the hydrostatic pressure of the blood in the

capillaries and the osmotic attraction of the blood for the surrounding fluids. The capillary pressure and the extra-vascular pressure, i.e., peritoneal pressure, must needs drive the fluid perhaps first into the interstitial spaces, and, secondly, into the blood channels, because extra-capillary or extra-vascular pressure will first come under intra-peritoneal (mechanical) pressure which will force fluids in the direction of least resistance. But the final regulation of intra-vascular and extra-vascular fluids doubtless chiefly comes under the laws of osmosis.

The rise in capillary pressure will increase the peritoneal lymph, but it will be thinner and hence the osmotic pressure of the blood will attract it vigorously to itself, thus bringing about a physiologic balance. Osmotic pressure holds an equilibrium between intra- and extra-vascular fluids. W. S. Lazarus-Barlow asserts that the blood serum of the ox, hare and sheep are in osmotic equilibrium at 37 degrees, with a Na. Cl. solution of 1.6 per cent. on the other side of the membrane. Also the greater the amount of proteids which a specimen of serum contains the more concentrated must be the solution of Na.Cl. on the other side of the membrane necessary to produce osmotic equilibrium. The reason of this is that proteids have large molecules and are not readily diffusible through fine membranes. A solution of sodium chloride may at first absorb the serum from the other side of the membrane, but finally become itself absorbed by the serum.

On the subject of the initial rate of osmosis we refer the reader to the excellent articles of W. S. Lazarus-Barlow. In his article on the initial role of osmosis Barlow makes the significant statement that it is impossible to state from a determination of their freezing points that one solution is hypertonic, isotonic or hypotonic as regards another solution of a different composition of pressure within the limits possible in the animal body. He experimented with glucose, Na.Cl. and urea. He states that in the case of prepared peritoneal membrane the initial rate of osmosis of glucose of Na.Cl. and urea is diminished by the pressure of albumen in the solution, even if that albumen be present only in small quantities. The initial and final rate of osmosis of the same substances are not the same.

Dr. J. B. Leathes, of London, England, made some excellent investigations in regard to the exchange of fluids between the blood and tissues. In his paper he considered the changes in the volume of the blood that are effected by injecting into the veins of an animal hypertonic, isotonic and hypotonic solutions of salt and sugar. Also he took into account the relations of osmotic pressure of the lymph to that of the blood serum, both under normal conditions and under abnormal conditions brought about by such injections. He then instituted a comparison of the rates of lymph flow induced by these means with the rates at which fluids



leave the blood vessels. A brief summary of Dr. Leathes' well conducted experiments may be noted in the following: Changes in the osmotic pressure in the blood are compensated with extreme rapidity by the transformation of fluid from tissue to blood or from blood to tissue when the kidneys are excluded from the circulation. There is no evidence that vessel walls play other than the part of a passive membrane in the interchange of fluids. They cannot be said to have the power of activity regulating the composition of the circulatory fluid.

The osmotic pressure of the lymph from the thoracic duct is always slightly above that of the blood. This slight difference is not effected by alterations in the osmotic pressure of the blood and is more easily accounted for by metabolism in the tissues than by any active function of the vessel walls. For further information the reader is referred to Dr. Leathes' valuable paper.

Osmosis plays a very significant role in the absorption of fluids from the peritoneum, as I am convinced from my own experiments on dogs and rabbits. But much credit is due to the extensive and suggestive labors of Dr. H. J. Hamberger, of Utrecht, Holland, in the subject of experimental osmosis on animals, for which I gladly acknowledge my indebtedness. From experiments it appears that serous fluid, from whatever origin, introduced into the abdominal cavity



FIG. 230.—(Author.)—Is drawn from a turtle's peritoneum near the gestating ova sac to show the endothelia reproducing or in a karyokinetic stage.

became absorbed. If the introduced serous fluid be isotonic with blood of the one on which the experiment is performed, it will remain so during the entire time of absorption. Isotonic solutions absorb very slowly or not at all, hence filtration must play the chief role in the absorption of such fluids. Hypotonic fluids introduced into the peritoneal cavity remain so until absorbed. Hamberger claims that hypertonic fluids introduced into the peritoneal cavity remain so until the absorption is completed, but some other force as filtration must come in to explain the process, for hypertonic solution under the law of osmotic pressure should become first isotonic and then hypotonic and become absorbed.

If there exists ascitic fluid possessing a higher osmotic pressure than the blood, there must exist some power which explains the condition. In such ascitic conditions during the absorbing of the lymph (separated from the blood by filtration) in increasing ascites more new fluid must enter, which exceeds the osmotic pressure of the blood and thus maintains the ascitic existence. In progressive ascites the abdominal fluid

possessing osmotic pressure to exceed that of the blood, a hypertonic solution is made isotonic with the blood by the law of osmosis. Experiments demonstrate that non-serous fluids, as Na.Cl. or sugar solution, injected into the peritoneal cavity become absorbed.

Hamberger claims that a 0.92 per cent. Na.Cl. solution is isotonic with the blood, as is also a 1.47 per cent. solution of  $\text{Na}_2\text{SO}_4$ , a 1.55 per cent. of  $\text{K. NO}_3$ , and a 7.95 per cent. of cane sugar solution. Now, if a Na.Cl. solution of 0.92 per cent. is isotonic with an animal's blood, when injected into such an animal's peritoneum its isotonic pressure will remain unchanged during the entire time of absorption. The same may be asserted of the above named salt solutions as for the Na.Cl. solutions.

If one injects into the peritoneum a hypertonic, e. g., 2 per cent. Na.Cl. solution, or one isotonic with it; a  $\text{Na}_2\text{SO}_4$  solution of 3.27 per cent. or a  $\text{K. NO}_3$  solution of 3.45 per cent. or a cane sugar solution of 17.7 per cent. it will become isotonic with the intra-peritoneal fluid, during absorption, or the same osmotic pressure with a 0.92 per cent. Na.Cl. solution and remain so until the absorption is completed (Hamberger). The above author also asserts that if one injects into the peritoneal cavity a hypotonic solution, e. g., a Na.Cl. solution of 0.5 per cent., or a solution isotonic with it as a  $\text{Na}_2\text{SO}_4$  solution of 0.735 per cent., a  $\text{K. NO}_3$  solution of 0.77 per cent., a cane sugar solution 3.97 per cent. it will become during absorption isotonic with the intra-peritoneal fluid or the same osmotic pressure as 0.92 per cent. Na.Cl. solution, and remain so until the absorption is completed.

During the residence of fluid in the peritoneal cavity they exchange constituents with the blood. Heidenhain, Orlow, Tubby and Starling were once inclined to add the power of vital cell forces to aid peritoneal absorption. Absorption after thermic and chemical injuries to the cell, as well as many hours after death, is an argument against such a view. Hamberger was inclined to think (1895) that imbibition and osmotic tension were sufficient factors in absorption to explain the phenomenon. But filtration and stomata are not so easily disposed of as it is to write the above sentences. Molecular and capillary imbibition is necessarily a limited process, limited to even the repetition of a few volumes, as any tissue will absorb more than it ordinarily contains and yet must be very circumscribed in absorbing fluids. If the imbibed fluid (lymph or blood vessels) is actively led away by the blood and lymph stream, imbibition could repeat itself by many volumes. In the dead, as the lymph and blood streams are stopped, imbibition is very limited. Absorption of peritoneal fluids is a physical process.

DOES THE PERITONEAL FLUID BECOME ABSORBED BY THE LYMPHATICS OR  
BY THE BLOOD VESSELS?

The paths of absorption of peritoneal fluids have been generally described, viz.: The absorption by the lymphatics and absorption by the blood vessels. If one will look over the history of the subject he will find that each path in turn has dominated writers according to their individual opinion or according to the state of medical science at the period of writing. Doubtless, absorption of peritoneal fluids may occur through both paths. The object of my own experiments was not only to observe whether blood vessels or lymph vessels absorbed the peritoneal fluids, but to study the mechanism of absorption; in other words, its histology and its physiology. My experiments extended over several years on the peritoneum but records here presented only of a limited number. Some of the other experiments will be alluded to in the discussion.

The plan of the experiments was to observe the method of disappearance and the rate of the disappearance of fluids from the peritoneum. If possible, the force which induces peritoneal absorption we tried to demonstrate. Finally, we endeavored to learn by exclusion whether peritoneal fluids passed directly into the blood vessels of lymphatics. The methods of exclusion were to ligate the left innominate vein which includes the thoracic duct (lower) or the right thoracic duct (upper), by ligating the right innominate vein. Sometimes both right and innominate veins (or both thoracic ducts) were ligated. We then endeavored to demonstrate the influence of ligating the (thoracic ducts) innominate veins, on the absorption of peritoneal fluids by placing potassium ferrocyanide in the solution in the peritoneal cavity and noting the time it required for the potassium ferrocyanide to travel from the peritoneal cavity to the bladder with ligated or non-ligated lymphatics. The traveling of the potassium ferrocyanide from the peritoneum to the bladder means that it passed perhaps into the lymphatics, then into the portal vein up to the right side of the heart, then into the lungs through the left heart, aorta, renal artery, kidney and down the ureter to the bladder. The whole circle from peritoneum to bladder with non-ligated lymphatics was completed by the potassium salt generally in 7 to 8 minutes. With ligated lymphatics it generally required from 25 to 30 minutes to complete the circle, i. e., a difference, say, of 20 minutes. The test to demonstrate that the potassium ferrocyanide had completed the circle from the peritoneum to the bladder was made by pressing on the animal in the region of the bladder, forcing out the urine and adding to it a solution of  $\text{Fe}_2\text{Cl}_6$ . When the slightest trace of ferrocyanide appeared in the urine, a drop of the solution  $\text{Fe}_2\text{Cl}_6$  being added to it produced a blue color. The animals employed in the experiments were



the dog, cat, rabbit and guinea-pig. The experiments on the dogs we performed before the students in the Harvey Medical College Laboratory.

Ligation of the lymphatics: 1, retards the absorption of peritoneal fluids; 2, it retards (about 20 minutes) the traveling of the potassium ferrocyanide from the peritoneum to the bladder. A necessity for the comprehension of absorption of peritoneal fluids is a knowledge of the lymphatic apparatus. It consists of tubular channels and interstitial spaces. The lymph channels lead the lymph fluid away from the tissues to terminate in the thoracic duct. The interstitial spaces, which term we shall choose instead of lymph spaces, are the laboratories of nourishment and the receptacles of effete matter. The numerous interstitial spaces are the numerous fluid laboratories where numerous cells functionate—nourish appropriate material for their existence, reproduce, expand and contract, in fact, where cells pass their lives. The fluid pabulum contained in the interstitial spaces furnishes food for cell existence and receives the products of effete life. The subperitoneal tissue passes an enormous and well-developed system of interstitial spaces and lymph channels. We can directly demonstrate that the lymph channels are lined with endothelial cells; in fact, some of the lymph tubes are formed of a single layer of the endothelial cells. We can also assert that endothelial cells line a vast majority of the interstitial spaces; perhaps we cannot demonstrate endothelial cells lining all of the spaces.

The interstitial spaces occupy vast and extensive areas in the subperitoneal tissue. The spaces communicate with each other so that an immense amount of fluid could be quickly accommodated in them, for the size of the interstitial spaces depends more on the amount of fluid they contain than on any anatomical circumscription. Injections of fluids into the subperitoneal tissue not only fill the interstitial spaces, but the lymph channels. The larger lymphatic channels are distinctly characterized by well formed valves located at short intervals along the channel. All the lymphatic channels of the peritoneum converge to empty into the thoracic duct, which in turn empties its contents into the left subclavian vein at the foot of the jugular. The valves in the lymphatic trunks prevent the retrograde flow of lymph, while the muscular action, chiefly, forces the lymph onward. Perhaps the constant muscular motion of life is the essential propeller of lymph, through both interstitial spaces and also valved lymphatic trunks. Any one can demonstrate the wonderful influence of muscular action on filling lymph channels by the injection of Berlin blue in fluid suspension into the peritoneum. By keeping a rabbit still the particles of Berlin blue will only partially fill the vast bed of lymph channels in the diaphragm, but by inducing rapid and continued action of the diaphragm and other abdom-

inal muscles, one can typically fill the numerous and large lymph vessels in the diaphragm.

To Ludwig and his pupils belong the credit of distinctly associating muscular action and lymph flow. Vast and numerous interstitial spaces exist in muscles whose natural activity induces lymph flow, located as they are in the midst of a large area of subperitoneal tissue. In most of the muscles and organs there exists a superficial and deep system of interstitial spaces and lymph tissues. An especially typical and double system of lymph channels and interstitial spaces exist in the diaphragm, held in close and intimate connection by vertical channels passing through the intertendinous spaces. All muscular motion, every breath taken, every step made, word uttered or song sung induces lymph flow. Of course, anesthetized animals lack in muscular activity, and hence will have much less lymph flow.

It is a very difficult problem to decide precisely whether the chief role of absorption be exercised by the lymph or blood vessels. Starling and Tubby injected colored material into the peritoneal cavity and found that the dye-stuff appeared in the urine five minutes later, whereas the lymph presented no blue color until twenty-five minutes after injection. This

rapid absorption must be accomplished by the blood current. The dye-stuff traveled a long distance in five minutes, from the peritoneum to the bladder. Experiments show that constant interchange of constituents occurs between various salt solutions in the peritoneal cavity and in the blood, so that peritoneal fluids of various composition soon become isotonic with the blood. As long as there is a difference between (intra-peritoneal) extra-vascular and intra-vascular fluids, so long will diffusive currents be set up, tending to equalize the differences.

An argument against the absorption of peritoneal fluids by the lymphatics is that during the absorption of a large quantity of peritoneal fluids the flow in the thoracic duct does not materially increase. This argument may be offset by saying that the vast and numerous interstitial spaces of the subperitoneal tissue can accommodate immense quan-



FIG. 231.--(Satterthwaite, 1881.) From mesentery of cat, silvered. Right portion denuded of endothelia. A. Branched cell with B intervening spaces. Left portion endothelial layer intact. C, C, Pseudo-stomata; D, D, nuclei; E, E, elastic fibres, drawn here with double contour. Observe that what Satterthwaite designates pseudo-stomata we have named stomata vera, for they occur at the common junction of several endothelial plates.

tities of fluid quickly by merely distending their collapsed walls. The absorbed peritoneal fluids would distend the subperitoneal interstitial spaces surrounding the blood vessels like connective tubes. Larger areas of distended interstitial spaces would really become peri-vascular fluid, resembling the small, rod-like vessels of the turtle's peritoneum, coursing through wide fields of lymph fluid. In such a condition the flow in the thoracic duct would slowly but materially increase later, as would also the excessive mechanic pressure in the interstitial spaces induce the concentric blood vessels to absorb considerable fluid by filtration. Under the above circumstances the lymphatics are really the primary paths of absorption of peritoneal fluids, the blood vessels are secondary.

In dropsical conditions of the peritoneum we are dealing with different matters. The ascitic fluid in the peritoneum is not far removed from the composition of blood plasma. The dropsical fluid, as noted by Starling, is only a little poorer in proteids. Hence the fluids on each side of the peritoneum are so much alike that no especial currents created by osmotic tension or diffusive currents are set up, for the dropsical fluid is almost identical with the blood plasma. Diffusion currents through the peritoneum are occasioned by fluids possessing widely different composition located on different sides, and the very difference in composition is what starts the currents, but diffusion currents do not produce a final absorption of the peritoneal fluid. Many experiments have been instituted to prove either the domination in absorption of peritoneal fluids by the lymphatics or the blood vessels.

Orlow, investigating in Heidenhain's Breslau laboratory and, doubtless, imbued by the opinions of his master, injected isotonic salt solution in the peritoneal cavity and found, as all have who experiment, that they absorb rapidly. I have noted the same effect almost two-score times in my own experiments. But Orlow concludes that because the lymph flow from the thoracic duct is not increased, or only slightly increased, it must be the blood vessels which accomplish the absorption of the peritoneal fluid. I cannot see sufficient proof for such a conclusion. To one who has studied microscopically the amphibian peritoneum or the human subperitoneal tissue, it would seem far more probable that the peritoneal fluid had rapidly passed into and become quickly accommodated by the innumerable vast areas and interstitial spaces of the subperitoneal tissue. Also, doubtless, Orlow, as well as his able chief, Heidenhain, are in error, for they assert that the absorption of peritoneal fluid is dependent on the "vital forces" of the endothelial cell lining the peritoneum and those of the capillaries. The fact that the peritoneum absorbs fluid even after death, and under chemie and ther-



mic injuries, is a sufficient death blow to "vital cell forces" in peritoneal absorption of fluids.

J. B. Leathes and H. J. Hamberger side with Orlow in the opinion that the blood vessels absorb the peritoneal fluids, but definite proof must be forthcoming, for it is not discernible in their valuable pamphlets, which all lie before me, through their courtesy. The fact is that such experiments as carried out by Hamberger, Orlow, Heidenhain and Leathes do not prove conclusively that the blood vessels are essential in the peritoneal absorption of fluids. I have performed many experiments on the peritoneum of animals of the above kind and do not see my way clear to the establishment of the blood vessels as the essential in peritoneal absorption of fluids.

So far, in a large series of experiments performed by myself, I am inclined to believe that the essential role in peritoneal absorption of fluids is performed first by the vast interstitial spaces of the subperitoneal tissue. The reason the lymph flow from the thoracic duct does not immediately increase is because the peritoneal fluid rapidly enters and distends the interstitial spaces, for the wide area of the interstitial spaces is naturally partially collapsed. It might be thought perfectly capable of easy determination which vessels absorb peritoneal fluids if both the upper and lower thoracic duct be ligated. But I can assure the reader that it is not. I have performed quite a number of experiments in regard to the absorption of peritoneal fluids, when both superior and inferior thoracic ducts were ligated, without being able to make definite decisions, yet so far I am inclined to say that the interstitial spaces perform the essential office of immediate and rapid absorption of peritoneal fluids, and that they are immediate storehouses of any excessive or emergency fluids of the peritoneum, but separated from it by perivascular or interstitial spaces of vast magnitude. With a microscopic knowledge of the vast and accommodating interstitial spaces, the elastic, distensible clefts, pouches or gaps in the wide bed of subperitoneal tissue, we can at a glance see what a considerable amount of fluid could be stored away without the slightest demand of the thoracic duct for aid. Colnstein, after performing similar experiments to those of Hamberger, Orlow and Heidenhain, concluded exactly opposite opinions, and that is that the lymphatics play the important role in the absorption of peritoneal fluids. To demonstrate why we can not make final decisions, even with both thoracic ducts ligated, in regard to the only path of absorption of peritoneal fluids, I can report quite a number of my own experiments where both thoracic ducts were ligated, yet, within an hour many particles of Berlin blue filled the vast bed of lymph channels and interstitial spaces in the diaphragm, while other investigators report the mediastinal glands well packed with colored granules under the same

circumstances. Lymph has passed up through the chest in spite of ligation of all the thoracic ducts. Other methods must be resorted to to decide the primary paths of absorption of peritoneal fluids. The lymph vascular system is an evolutionary addition to the blood vascular system, a secondary vascular system, and would be naturally looked to as the immediate accommodater for excessive fluids within its own borders, i. e., in the peritoneum. The whole system of interstitial spaces being directly connected with each other in the subperitoneal tissue, the peritoneal fluids absorbed may easily pass from chamber to chamber until a large amount of fluid is stored away.

Another method to demonstrate the paths of peritoneal absorption is by bleeding an animal and noting the amount of solids in the blood at each successive bleeding. If the solids become diminished at each successive bleeding it must be due to dilution of the blood serum. Starling gives several examples, of which I will quote three:

1. Dog, 11.40 kilos. Solids in serum, 7.72 per cent. The dog was bled 220 c.c.m. Thirty minutes later the solids in the blood serum became diminished to 7.14 per cent.

2. In another experiment the solids were 6.98 per cent. The dog was bled 200 c.c.m. when the solids were 6.57 per cent. After further bleeding of 100 c.c.m the solids were 6.37 per cent.

3. Dog, 6.5 kilos. Bled 150 c.c.m., which reduced the solids 7.77 per cent. to 6.47 per cent.

In the above three experiments the successive bleeding showed a continual diminished condition of the solids in the serum, which means that the blood becomes more watery, and it also means that the blood acquired its fluid to dilute itself from the fluid in surrounding lymphatic spaces. This is an argument by Starling, produced to show that the blood vessels absorb the peritoneal fluid. But it must be remembered that by rapid bleeding the equilibrium of osmotic tension is disturbed and, besides, the factor of filtration (mechanical pressure) would be brought into aid in inducing fluid to flow to the depleted vessels, so that this simply means that the blood vessels do absorb peritoneal (interstitial spaces) fluids, not that the blood vessels are the essential actors in the play. It is quite evident that the thinning of the blood and decrease in the per cent. of solids after repeated bleedings is due to the absorption of thinner fluids from adjacent interstitial spaces, because the blood will thin about the same after bleedings if the thoracic duct is tied or if its fluid be carried away by a canula. So that the thinning of the blood is not due to lymph being poured into it from the thoracic duct. But this whole argument, arising from repeated bleedings and decreased solids in the blood serum, does not seem to me impregnable, for both capillaries of blood and lymph are imbedded in the

wide lymph or interstitial spaces. These capillaries are only separated from the lymph sinuses by a single layer of endothelial membrane, doubtless provided with stomata, and thus prepared to receive or give up fluids. It only shows that the blood capillaries are a factor in the absorption of peritoneal (interstitial) fluids. The amphibian peritoneum (especially the turtle) will show well that the blood and lymph capillaries do not open into each other directly, but there is a lymph space, an interstitial space, or several, or a lymph sinus existing between the two systems. This intercapillary space is where the life of the cells is preserved, nourished, reproduced and functionates in this vital pabulum. It is admitted on all hands that rise of blood pressure produces, filtrates, more lymph. In the same way, the taking away of fluid from the blood vessels insures, by mechanical pressure, filtration, a flow of fluid from the lymph spaces actually surrounding and bathing blood vessels possessing only a single layer of stomatized endothelial membrane. Yes, this argument proves conclusively that blood vessels absorb peritoneal fluids, but it does not prove that blood vessels play the essential role. However, so far as I am aware all experimenters admit that blood vessels absorb peritoneal fluid to some extent. In studying the paths of peritoneal absorption, I am convinced that more attention should be paid to the system of interstitial spaces which is connected on the one hand by the blood vascular channels and on the other hand with the lymph vascular chan-

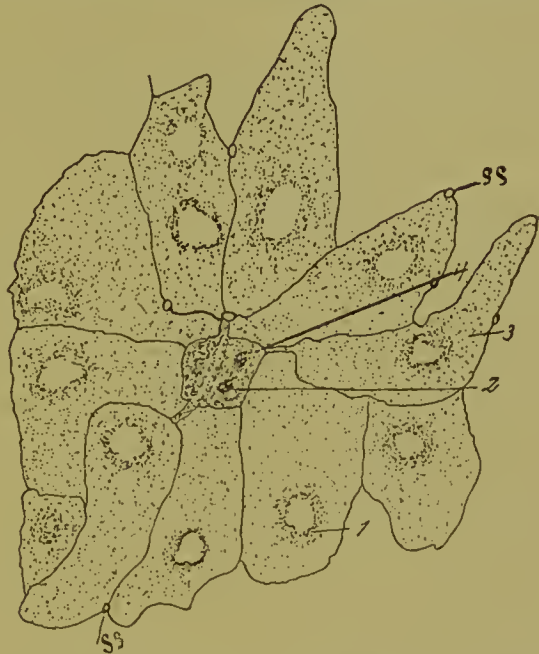


FIG. 232. — (Author.) Sketched from eisterna lymphatica magna of frog (oe. 2, obj. 8a. R.). The frog was opened and the parts stained in situ with one-half per cent. solution of silver nitrate for about four minutes. The large irregular endothelial cells are plainly marked off by dark lines which are stronger according to the length of time the silver remains on and the kind and strength of sunlight. 1. Nucleus of endothelial cell; 3, points to the cell itself, while quite near it is an open stoma spurium. Several others (SS) may be also observed. 4, points to a stoma verum in which there appear to be three young granular many-faced cells; 2, points to the nucleus of one. One granular cell of the stoma verum does not show a distinct nucleus—only thickened granules. The stoma verum is shut. The Ag.  $\text{NO}_3$  did not brown the nucleus, but that may be due to the elevated condition of the nucleus and also liquid albuminous substance in more abundance at the junction of the cells. Observe that the nucleus is excentrically located. It was aimed to draw the cells as nearly natural as possible in all their relations.



nels. We may say the lymph spaces, the clefts and the gaps lying between tissue elements are fed on the one hand by tubular channels, the blood capillaries. They are drained on the other hand by tubular channels or trunks. Both systems of tubular channels, the lymph and blood capillaries, have many common properties: As (a) they are both tubular, having a single layer of endothelia for a wall. (b) The endothelial layer or wall of both capillary systems possesses an interendothelial space which has common properties. Through this interendothelial space passes the fluids. (c) The blood and lymph capillaries have definite directions for their current. (d) Both tubular systems may possess elastic and muscular fibres and (e) with a nervous apparatus, may control the volume of either current or the calibre of either tube.

The two systems of supplying and depleting tubes, possessing much in common, are very different from the system of interstitial spaces which are indefinite in size, shape and location, with no such definite direction of currents. The fields of nourishment in the body are the interstitial spaces, while the tubular system, the blood vascular and lymph vascular channels, are merely the routes of transportation, as suggested by Adler and Meltzer. The function, then, of the interstitial spaces is nourishment, to float to cells what they need to live, and to float away from them effete material. The function of the tubular vascular system is transportation, that of the interstitial spaces is nourishment. The various tissues in various organs require different material for nourishment and, of course, give off different kinds of effete matter. Hence, in this interstitial space will be found, 1, the fluid transuded from the blood vessels; 2, plus the effete material from cell activity; and 3, minus the fluid required for the cell nourishment.

The thoracic duct itself not only carries all these various constituents of life and death, debris, of the interstitial spaces, but also the lymph from the intestinal canal. Allowing this wider and more significant position to be the interstitial spaces, we may more clearly know their office. Their anatomical limitation and their physiologic function becomes manifest by experimentations. The spaces become filled with Berlin blue and their associated channels become loaded in carrying it off. It is natural to consider that a large amount of lymph flows through the superior and inferior thoracic ducts, but it must be remembered that the two thoracic ducts are not the only outlet for the interstitial fluids. Interstitial fluids pass out by way of—

- (a) The salivary glands.
- (b) The intestinal mucosa.
- (c) The mammary glands.
- (d) The tears.
- (e) The sweat glands.

(f) The genito-urinary tract.

(g) From chemical action.

If we take all the secretions together they will doubtless form a much larger demand on the supply than do the thoracic ducts. Starling carried out some experiments to prove that the blood vessels were the chief absorbents of the extra-vascular fluids or the fluid in the interstitial spaces. His experiments were carried out on amputated limbs. He made one limb dropsical or oedematous by injecting into it isotonic salt solutions. He sent through the dropsical and non-dropsical limbs defimbriated blood and found that as the defimbriated blood passed through the dropsical limb it collected some of the old dropsical extra-vascular fluids, showing that the blood vessels would absorb interstitial (peritoneal) fluids. But, again, it seems to the author that such proof is insufficient to dignify the blood vessels as the chief absorbents, because we know from repeated experiments that increased intra-capillary pressure increases lymph filtration into the interstitial spaces and the reverse, so that intra-vascular and interstitial pressure have direct relations with each other to a certain extent only. It is evident from the structure of a capillary wall that transudation to a certain extent depends on intra-capillary pressure. As the intra-vascular pressure rises transudation increases, and the reverse. But this view only includes filtration or mechanical pressure, while osmosis, stomata, imbibition, etc., are left out. Transudation into and absorption from the peritoneal cavity demand more than filtration or mechanical pressure to explain all the phenomena.

From writers and investigators we learn that the fluid pressure in the interstitial spaces (peritoneum) is very low. Heidenhain at first denied that lymph was directly returned to the blood vessels, but later he asserts that the blood vessels are the essential (wesentliche) paths of absorption of peritoneal fluids. From his articles I was unable to learn why he changed his opinion. There is a theory extant, which, so far as I am aware, was started by Prof. Thoma while he was assistant to Prof. Arnold, in Heidelberg. It is that the endothelial cells of capillaries have a secretory power. I do not think that this secretory power can be referred or transferred to the peritoneal endothelia as endowing them with an absorptive or "vital" power. Our view of the subject of the disappearance of fluid from the peritoneum is that it first enters the interstitial spaces and, secondly, the blood vessels, that the chief factors are osmosis, filtration, stomata and imbibition. That the lymphatic channels are the real drains or depleters of the interstitial spaces, the sewers.

After the excessive peritoneal fluid has entered the interstitial spaces, what becomes of it? We observed previously that any excess of fluid in

any portion of the tissues was bound to move in some direction so that a physiologic balance would be assumed. First, the excessive fluid in the interstitial spaces can equalize matters by spreading wider in other interstitial spaces. Second, it can pass into the lymphatic channels. Third, it may pass into the blood vessels. Perhaps osmosis tells the chief story of why the answer to Prof. Heidenhain and W. N. Orlow, his pupil, that the thoracic duct does not increase its flow during rapid absorption of peritoneal fluid is that the fluids disappear first into the interstitial spaces. However, I cannot see sufficient proof from the works of Heidenhain and Orlow that the entire fluid absorbed has passed directly into the blood vessels.

That the fluid absorbed from the peritoneal cavity does not enter the lymphatic channels as they claim is no proof that it does not enter the interstitial spaces, and also it is no proof that it enters the blood vessels. If the blood vessels do absorb the peritoneal fluid they would have the advantage over the lymphatic vessels in that the blood current would rapidly remove all particles to make room for new ones.

H. J. Hamberger makes the statement in his famous pamphlet that since the absorption of peritoneal fluids proceeds almost as rapidly and completely after ligation of the thoracic ducts, that hence it follows per exclusion, that if the blood vessels are not exclusively responsible for the absorption, they are answerable for the chief role. To me the proof of the above assertion is insufficient. I am aware of the impressive fact that in my experiments the material injected into the peritoneal cavity gained the bladder in seven minutes, but it was in small quantities and did not prove that the blood vessels played the chief role in the absorption of the peritoneal fluid. Doubtless the vast area of interstitial spaces was trying to store away the excessive interstitial (peritoneal) fluid, and during the process some of the material passed through their endothelial capillary wall. I noted very carefully in the experiments that the urine became a deep blue color, always very gradually, requiring about 20 to 30 minutes. If the blood vessels were the exclusive path of the absorbing peritoneal fluid the urine would show deep blue reaction much more quickly, as the blood stream is a very rapid one. Hamberger also claims as proof an experiment which I did not carry out, that after ligation of the renal arteries the osmotic tension and peritoneal absorption are deficient. This is only what we should expect, for the kidneys are the chief outlet for circulatory fluids, and cutting off their action suddenly would unbalance the whole blood and lymph vascular systems. To arrest totally the renal circulation (and hence secretion) is to distort physiologic action into pathologic process. It must be remembered that we are dealing in all of our experiments



with final rates of osmosis and not initial rates—a subject in which J. B. Leathes has done valuable service.

The whole subject of what power induces absorption of peritoneal fluids should be re-cultivated by extensive experiments carefully planned. We are gradually discovering factors which in time reveal new ones and have advanced to the stand that absorption of peritoneal fluids is, to say the least, a complicated process. We can, however, hold the facts of osmosis and filtration as well founded factors. We can look to the stomata and imbibition as limited but unsettled factors. We may look to “vital” forces in the cells as very unsettled and limited, though advocated by such names as Heidenhain, Orlow, Starling and Tubby.

Mechanical pressure, in general, is equivalent to filtration. Absorption of peritoneal fluid is a physical process. Doubtless, as a fact, both blood and lymph vessels play the role of absorption of the peritoneal fluids, but we so far incline to the opinion that the interstitial spaces are the primary and chief paths.

Starling and Leathes repeated the experiments of Orlow and Hamberger, but frankly admit that they could not decide which were the channels of absorption (1896). Cohnstein, after experiment and a slow rise of lymph flow from the thoracic duct, concluded that the lymphatics were the sole channels of absorption. Starling again asserts (1896) that he has made a number of experiments with ligatures in various ways about the thoracic ducts (and innominate vein), but could never be certain that the lymphatic pathways had been blocked by his ligatures. I found in all my experiments, as also Starling notes, that though the thoracic ducts right and left were ligated, the Berlin blue (colored granules) passed up into the chest glands and lymphatics. Curious enough, later, Starling had frankly admitted that by experiments he could not decide on the channels of absorption. He concluded his article with the astonishing remark that “salt solutions, isotonic with the blood plasma, can be and are absorbed directly by the blood vessels,” the proof of which is not quite evident. He also claims that the proteids of the tissue fluids when not used up in the tissues themselves are probably absorbed mainly, if not exclusively, by the lymphatic system. It has been shown by W. S. Lazarus-Barlow that the initial role of osmosis of glucose, Na.Cl. and urea in the case of prepared peritoneal membrane is diminished by the presence of albumen in the solution, even though the albumen be in small quantities. Heidenhain and Orlow, his pupil, lay considerable stress on the fact that in the peritoneal cavity salt may be taken up from fluids containing a smaller percentage of this substance than does the blood plasma, and they regard this absorption as pointing undoubtedly to an active intervention of liv-

ing cells in the process. J. B. Leathes and Ernest H. Starling make the following answer to this point:

"It is evident that neither the raising of the percentage of a salt in any fluid above that of the same salt in the plasma nor the passage of a salt from a hypotonic fluid into the blood plasma can afford in itself any proof of an active intervention of cells in the process."

We wish to record emphatically that ligation of the thoracic duct in our experiments reduced the amount of peritoneal fluids absorbed in a given time. Adler and Meltzer agree to the same in their labors. The above recorded fact is not so easy of interpretation. It may be that as the lymph is not carried away from the interstitial spaces that they are occupied by the old accumulating interstitial fluid, which does not give time for the fresh. But this will hardly account for the large amount of peritoneal fluid rejected in the absorption by the ligation of the thoracic duct. Further than the statement that ligation of the thoracic duct diminishes the amount of fluid absorbed from the peritoneum the facts do not fully warrant. Beyond this statement it is simply debatable grounds whether the lymphatics or the blood vessels accomplish the chief role. Adler and Meltzer showed by experiments that the effect of strychnine on the system was observed much quicker with open lymphatics than with closed. It is evident from actual experiment that strychnia affects the system much later with ligated lymphatics, and it is fairly clear from experiments that with ligated lymphatics salts require about twenty minutes longer to travel from the peritoneum to the bladder. In all probability this retardation is due to obstruction of the thoracic duct and consequent damming by the ligature. From such results we are inclined to ascribe the chief role in the absorption of the peritoneal fluid to lymphatics or, rather, interstitial spaces. We performed experiments on quite a number of dead animals by injecting into the peritoneum various kinds of fluids. In the dead peritoneal absorption is accomplished just the same as in the living, but not with quite the same rapidity. It appears to me that the probability is even greater in the dead than in the living, that the interstitial spaces are the primary and chief path of peritoneal absorption, for in the dead the blood current fails absolutely and the lymph current fails shortly after death. The chief propellor of the lymph stream, which is muscular action, fails in the dead. Vital action of cells fails shortly after death. The chief factors left to induce peritoneal absorption are osmosis and filtration.

It seems, however, that the quantity of flow from the thoracic duct is so limited that but little account of that flow can be considered in the rapid absorption from the peritoneum of so large a quantity of fluid. In the dead the peritoneal fluid will enter the interstitial spaces of the

subperitoneal tissue only. It will not enter the lymph or blood capillaries, as there is no current in them to remove old molecules to give place to new ones. The absorbed fluid will also be limited to the superficial portions of the subperitoneal tissue as respiratory, and all other muscular movements fail to aid in distributing it over other areas of interstitial spaces. This accounts for what experimenters characterize as imbibed tissue in the dorsal peritoneum of rabbits and dogs in whose peritoneal cavity fluids were injected while dead. In my experiments it was rare to note definite imbibed or oedematous tissue in the living animal.

We will here note some of the works of Adler and Meltzer, of New York, who performed a number of experiments to discover the path by which fluids are carried from the peritoneal cavity into the circulation. Their article was published in the *Journal of Experimental Medicine*, Vol. I, No. III. Their experiments were done carefully and systematically and is the first record of its kind in this country, and to which we are glad to give due credit. However, they confine their references to but a few (6) writers of recent date. The vast labors, extending over thirty years, begun by Von Recklinghausen and carried on by his pupils, Pia Foa and Radjewsky, by Ludwig and his pupils, Schweigger-Seidel, Dogiel and Dybkowsky, by Lawdowsky, His, Frye; by Chrzonszczewsky and his pupil, Affannasiew as well as Auerbach, Bizzozero, Maffucci, Ranvier, Kumdrat, Arnold, Kolossow, Muscatello, Oedmansson, Dubar and Remy, Klein, Burdon-Sanderson, and many others are passed over almost in silence. In fact, these writers confine their references almost entirely to Starling and Tubby, Orlow, Heidenhain, Cohnstein and Hamberger, each of whose labors we will speak of in this volume.

The experiments of Adler and Meltzer consisted in—1, determining whether in absorption of materials the peritoneum of the normal animal acted by way of the lymphatics or by way of the blood vessel; 2, determining which path the fluids assume as they pass from the peritoneum when the jugular vein (or veins) were ligated, i. e., whether by blood vessels or lymphatics. 3. Determining the path which peritoneal fluids take when the innominate vein (or veins) were ligated, which means when the thoracic duct is ligated. The method they assumed was to inject into the abdominal cavity a solution of potassium ferrocyanide dissolved in a 1 per cent. solution of sodium chloride. They then tested the urine about every ten minutes by pressing on the bladder and adding to the urine a little perchloride of iron whence the characteristic blue color of ferric salts presented. The urine reaction is delicate and accurate, for even in a dilution of 1 to 40,000 the addition of perchloride of iron will bring out the fine blue color. The lymphatics were also tested with the potassium ferrocyanide and ferric



chloride by dipping cotton wetted in the salt in the right or left thoracic cavity whence it would show by the chemical action, blue color, whether the lymph vessels had carried the fluid. The general result of their experiments with the chemical salts was that introduction of the salts into the urine of the bladder (which means into the blood vascular system) is by way of the lymphatics. Ligation of the thoracic duct in general retarded very much the entrance of the salts into the urine. Again, the Adler and Meltzer experiment on the peritoneal cavity with strychnine and the general results confirm the other experiments. The result of the strychnine on the animal was retarded by ligation of the left innominate vein (i. e., the thoracic duct). The result of the strychnine in the experiments was observed by the tetanic outbreaks of the animal. As we believe that the most accurate and practical views of the physiology of the peritoneum will be acquired by the observation of experiments, we will append and discuss some of the experiments of Adler and Meltzer.

Experiment 71. Rabbit A, 1,750 grammes, both innominate veins ligated. Rabbit B, 1,750 grammes, both external jugular veins ligated. Injected into the abdominal cavities of each 0.6 milligramme of strychnine at 4:19 p. m. No effect. At 4:29, 0.3 milligramme of strychnine was injected again into each; eight minutes, at 4:37, B had a characteristic opisthotonos; 4:44, no convulsions yet in A; added 0.3 milligramme of strychnine; at 5.8 added again 0.4 milligramme of strychnine; characteristic tetanus in A at 5:20. This means that in the rabbit without the lymphatics the strychnine took effect forty-three minutes later than in the normal rabbit, and that with a dose nearly twice as large.

Experiment 75. A, female rabbit, 1,460 grammes, both innominate veins ligated. B, female rabbit, 1,430 grammes, both external jugular veins ligated. Injected at 4:59 p. m. into the abdominal cavity of each rabbit one cubic centimeter of a five per cent. solution of potassium ferrocyanide. Test of urine after ten minutes, negative in both. At 5:18 injected again into both 0.5 cubic centimeter of same fluid. At 5:29, Prussian blue reaction in the urine of B; the urine of both tested every ten minutes; B retained the positive reaction. Urine of A shows for the first time the reaction of Prussian blue at 6:6. This means that in the rabbit without the lymphatics the potassium ferrocyanide reaches the urine thirty-seven minutes later than in the other rabbit. The same rabbits were then used for an experiment with strychnine. At 6:14 0.8 milligramme of strychnine was injected into each; B succumbed to a typical tetanus at 6:22; no convulsions in A. At 6:44 again 0.8 milligramme of strychnine injected into A. Tetanus at

6:55. The second part of the experiment gave results confirmatory of the first.

Experiment 75. A, male rabbit, 1,266 grammes, both innominate veins ligated. B, female rabbit, 1,280 grammes, both external jugular veins ligated. At 4:30 injected into A 0.9 cubic centimeters of five per cent. potassium ferrocyanide, and at 4:33 injected in B 0.7 cubic centimeter of same solution. At 4:43 no reaction in the urine of either. At 4:48 injected into each again 0.5 cubic centimeter of same solution; at 5:17, Prussian blue reaction in the urine of B, absent in the urine of A. Urine of A tested every ten minutes. Till 6:14 no reaction. At 5:35 a piece of absorbent cotton pressed into the right thoracic aperture in A, near ligature of right innominate vein, where some drops of lymph seem to collect, showed the Prussian blue reaction. No reaction in left aperture, where the tissue seemed to be dry. At 6:18 the testing of urine was discontinued and rabbits used for strychnine—large doses. Again a difference in favor of B, though not as great as before.

Autopsy of A: Distinct reaction of Prussian blue all over the abdominal cavity, except within the bladder. Autopsy of B: No reaction of Prussian blue in the abdominal cavity; present in the urine. In this experiment it looks as if very little, if any, absorption had taken place in rabbit A, with the ligated lymphatics.

Experiment 77. Rabbit A, 1,140 grammes, both innominate veins ligated. Rabbit B, 1,080 grammes, both external jugular veins ligated.

At 4:41 injected into A 0.9 cubic centimeter of five per cent. potassium ferrocyanide, and into B 0.7 cubic centimeter of the same fluid. At 4:52 test of urine of both negative. At 4:55 injected again into A 0.6 cubic centimeter, and into B 0.5 cubic centimeter of same fluid. At 5:37 the urine of B showed positive reaction of Prussian blue. Testing urine of A every ten minutes continued until 6:17, but no reaction. At 5:55 there was a positive reaction on cotton dipped in right aperture of A; no reaction from left. At 6:30 animals used for strychnine with same results as in the other experiments.

Experiment 84. Rabbit A, 2,180 grammes, both innominate veins ligated. Rabbit B, 2,150 grammes, both external jugular veins ligated. Both holders kept slanting; heads higher. At 3:56 injected into each 1 cubic centimeter of potassium ferrocyanide. Urine tested every ten minutes. At 4:20 in the urine of B a positive Prussian blue reaction; testing continued every ten minutes. At 4:55 first positive reaction in urine of A. Right thoracic aperture shows positive reaction of Prussian blue at 4:30, no reaction in left aperture. At 5:20 animals used for an experiment with strychnine, with the same characteristic results as those reported above.

These experiments from Adler and Meltzer show that the ligation of either right or left thoracic duct retards the appearance of the potassium ferrocyanide in the urine. It may be observed that even the right innominate vein (i.e., the right thoracic duct) being ligated, it retards the absorption from the peritoneal cavity, although the influence of the right thoracic duct is not so great as the left. The experiments show quite conclusively that ligation of the thoracic ducts (innominate veins) retards the absorption of peritoneal fluids, but the ligation of the thoracic ducts does not prevent the final entrance of peritoneal fluids into the general circulation. If, however, time sufficient is allowed to elapse after both the thoracic ducts are ligated, the solution from the peritoneal cavity will appear finally in the urine. This may be interpreted that the blood vessels absorbed the peritoneal fluids directly without the aid of the lymphatics. Yet, may it not be assumed that all the fluid (lymph) of the peritoneal cavity does not pass through the thoracic duct? Of course, a chief cause of lymph movement is the excess of intra-capillary pressure over extra-capillary pressure. If intra-capillary pressure is high there will be a considerable lymph secretion and also an increase of lymph flow. It is a principle of mechanics that fluid will flow in the direction of least resistance. Now, the lymph streams are derived from the capillaries because the intra-capillary pressure is higher than the lymph in the interstitial spaces, so that the stream of lymph will be away from the capillaries and not toward them. For this reason the fluids in the peritoneal cavity (in the interstitial space) will rather flow toward the thoracic duct than toward the capillaries whence it arose and where the pressure is high. An animal with ligated lymph ducts is, however, in an abnormal state, and as soon as the ligated ducts are full the pressure will need to assume a new direction. With the thoracic duct ligated the lymph fluid in the interstitial spaces will gradually assume a pressure as high as the intra-capillary blood pressure. With continued ligation of the thoracic duct the interstitial fluid pressure will eventually become higher than the intra-capillary pressure, and then the lymph fluid, interstitial fluid or fluid in the peritoneum will of necessity pass through the capillary walls into the circulation. It will be a mechanical necessity from filtration. But naturally, primarily, the path by which peritoneal fluids pass into the system is by way of the lymphatics—secondarily, by the blood vessels. Of course, with fluids in both interstitial space (peritoneum) and blood vessels of necessarily different density, osmosis (i. e., the tendency of a thinner fluid to flow toward a thicker) must be considered. The meaning of filtration is purely mechanical. A fluid filtrates, percolates or strains through any membrane or sieve where there is really a porous or perforated (preformed opening) condition. (Diffusion is a word



without definite and precise meaning in regard to structures, physical or chemical action, and we hence discard its use.)

The kinds of salts employed in the peritoneum, whether isotonic, hypertonic or hypotonic, must also be considered in the question whether peritoneal fluids enter the circulation by way of the lymphatics or by way of the blood vessels. We must acknowledge respectable investigators on both sides of the question—which can only be settled by experiment.

Experiment 81A. Male rabbit, 1,920 grammes; right jugular vein ligated. At 9:37 p. m. injected into peritoneal cavity 1 cubic centimeter of potassium ferrocyanide; at 9:59, twenty-two minutes after injection, Prussian blue reaction in the urine.

Experiment 81B. Female rabbit, 2,100 grammes; right innominate vein ligated; at 10:29 p. m. injected into peritoneal cavity 1 cubic centimeter of potassium ferrocyanide. At 10:44 0.5 cubic centimeter of same solution was added. First reaction of Prussian blue in urine appeared at 11:20 p. m., fifty-two minutes after first injection and thirty-two minutes after second injection.

Experiment 81C. Male rabbit, 1,560 grammes; left innominate vein ligated. At 9:58 p. m. injected into the peritoneal cavity 1 cubic centimeter of five per cent. potassium ferrocyanide; urine tested every ten minutes. First Prussian blue reaction at 11:8, seventy minutes after injection.

Experiment 83A. Male rabbit, 1,430 grammes; both jugular veins and left innominate vein ligated; thoracic duct apparently torn; whitish fluid oozing. At 5:40 p. m. injected 1 cubic centimeter of five per cent. potassium ferrocyanide; at 5:51 p. m. injected an additional 0.6 cubic centimeter of same solution. The whitish fluid in left aperture showed at 5:50 a positive Prussian blue reaction. At 6:34 the first positive Prussian blue reaction appeared in the urine fifty-three minutes after first and forty-three minutes after second injection. Lymph of thoracic duct showed Prussian blue reaction after injection.

Experiment 83B. Male rabbit, 1,160 grammes; both jugular veins and right innominate vein ligated. Injected into peritoneal cavity at 5:40 p. m. 1 cubic centimeter of potassium ferrocyanide. At 5:51 injected an additional 0.6 cubic centimeter of same solution; first Prussian blue reaction in urine at 6:18, thirty-eight minutes after first and twenty-seven minutes after second injection.

The foregoing experiments indicate that strychnine affects the rabbit with tetanic convulsions much earlier in the one with no obstruction in the thoracic duct. The thoracic duct must then be the agent to first carry the strychnine into the system, i.e., it passes from the peritoneum into the circulation by way of the lymphatics.

It may be noticed in these experiments, as almost every experimenter notes, that the most accurate physiologic knowledge is obtained by using small doses. Large doses so shock structures and function that we cannot differentiate so well the various manifestations of function. In my own experiments in the abdominal cavity I found the smallest amount of any material injected into the peritoneum which would arouse manifest function was entirely the safest indication of distinct function. These experiments show: 1, that if the thoracic duct is ligated it retards considerably the time of passage of fluids from the peritoneal cavity into the circulation, and, 2, that the path that fluids assume to travel from the peritoneal cavity to the circulation is primarily the lymphatics, and secondarily the blood vessels.

The experiments on the peritoneum with strychnine, depending on a tetanic outbreak in the animal, may be objected to on the ground that various rabbits differ as to their susceptibility to strychnine. This may be obviated by employing rabbits from the same region, fed on the same food and living under the same environments. But no such objections may be raised against the use of the potassium ferrocyanide. Adler and Meltzer claim that the Prussian blue reaction of the urine was constantly earlier in the rabbits whose thoracic duct was not tied. Objections against the use of potassium ferrocyanide will not likely be raised from any peculiar excitability of the animal or even irritability of the salt in the peritoneum, nor is it at all probable that the salt produced constitutional disturbances which would diminish or delay urinary secretion. Besides, it is noted in the experiment that in the rabbits with ligated thoracic duct, if the outbreak of tetanus was late from strychnine in the same rabbit, the appearance of the Prussian blue reaction of the urine was also late. Both kinds of experiments then go to show that the fluids of the peritoneum primarily travel through the lymphatics to gain the circulation. Another important factor in the experiments was that in the rabbit with ligated thoracic duct (innominate vein) Prussian blue reaction occurred all over the peritoneal cavity, and especially no Prussian blue reaction could be obtained in the urine. But in the rabbit with open thoracic duct no Prussian blue reaction could be obtained in the abdominal cavity, yet a positive blue reaction could be obtained in the urine. This shows that with open lymphatics peritoneal absorption is free while with closed lymphatics (ligated thoracic duct) peritoneal absorption is slow, retarded or absent to tests.

Experiment 31. Female rabbit, 1,960 grammes. Injected into abdominal cavity 100 cubic centimeters of 0.75 per cent. sodium chloride; killed after forty minutes; collected from peritoneal cavity 65 cubic centimeters; absorbed 35 cubic centimeters.

Experiment 32. Female rabbit, 2,100 grammes; right innominate

vein ligated; injected 100 cubic centimeters of 0.75 per cent. sodium chloride; killed after forty-five minutes; collected again 87 cubic centimeters; absorbed 13 cubic centimeters.

Experiment 33. Female rabbit, 1,920 grammes; left innominate vein ligated; injected 100 cubic centimeters of same solution as before; killed after forty minutes; collected 81 cubic centimeters; absorbed 19 cubic centimeters.

Experiment 34. Female rabbit, 2,400 grammes; right and left innominate veins ligated; injected into the abdominal cavity 98 cubic centimeters of the same solution; killed after forty minutes; collected 86 cubic centimeters; absorbed 12 cubic centimeters.

We will here quote four more of Adler's and Meltzer's experiments to illustrate the physiology of the peritoneum.

Experiment 31 shows without ligation of lymphatics absorbed 35 cubic centimeters.

Experiment 32 shows with ligation of right innominate vein absorbed 13 cubic centimeters.

Experiment 33 shows with ligation of left innominate vein 19 cubic centimeters.

Experiment 34 shows with ligation of right and left innominate vein 12 cubic centimeters.

In the above four experiments 100 cubic centimeters of 0.75 per cent. Na.Cl. solution were first injected into the abdominal cavity and allowed to remain 40 to 45 minutes before the rabbit was killed. They show that ligation of the thoracic ducts (right or left) diminishes the rapidity of peritoneal absorption about one-half, and the ligation of both thoracic ducts (i. e., both innominate veins) diminishes the absorption about two-thirds. It is, at least, an indicator that the lymphatics play an important role in the absorption of fluids from the peritoneal cavity. It is an especial feature to note that in experiments 32 and 33 the ligation of the right thoracic duct exercised more influence in retarding peritoneal absorption than the ligation of the left. There might have been in these subjects of the experiments an abnormally large right thoracic duct or some abnormally connected branches. The density of fluid injected into the peritoneal cavity has considerable influence as to the quantity which will be absorbed. Under ordinary conditions, with an average sized rabbit, by injecting three ounces of a 0.75 Na.Cl. solution in the abdominal cavity, some over one-third is absorbed in 40 minutes. Starving the rabbit for twenty-four hours or not starving him seems to have but little influence on the peritoneal absorption, as may be illustrated by the two following experiments from Adler and Meltzer.

Experiment 18. Female rabbit, 1,520 grammes; starved for twenty



hours; injected 98 cubic centimeters of 0.75 per cent. sodium chloride; killed after forty minutes; collected 61 cubic centimeters; absorbed 37 cubic centimeters.

Experiment 28. Male rabbit, 2,400 grammes; not starved; injected 100 cubic centimeters of 0.75 per cent. sodium chloride into peritoneal cavity; killed after forty minutes; collected 65 cubic centimeters; absorbed 35 cubic centimeters.

Same difference in experiment 18 and 28 may be noted, for the smaller rabbit, starved, absorbed more fluid from his peritoneum than the much larger and non-starved rabbit. It may be noticed that (100 centimeters) 3.3 ounces is a large dose to inject into a rabbit. I found in experiments that much more natural results were observed with smaller quantities injected into the abdomen, say 4 drams for a 4 pound rabbit, i. e., a dram to a pound. By weight of the animal 1 to 50 or 1 to 75 are good standards. In the experiments of peritoneal injections one must take into account the kind of salt used, the density of the fluid, the temperature, the quantity and the condition of the animal. Rabbits differ considerably in the power to absorb peritoneal fluids. But in my experiments nearly always the whole fluid was absorbed in eight to twelve hours (if allowed to remain that long), yet with quite different appearances of the peritoneum. In some cases the peritoneum appeared more pathologic than in others; in some rabbits, oedema of the subendothelial tissue was a prominent symptom, and in some animals in twelve hours the whole colored solution appeared to have been absorbed with a reaction of lymph pouring into the peritoneum. In the following six experiments of Adler and Meltzer on rabbits, by injecting 100 cubic centimeters of a 0.75 per cent. Na.Cl. solution and killing 40 minutes later it may be observed how variable is the individual capacity for peritoneal absorption.

Rabbit, 1,700 grammes, absorbed 25 cubic centimeters in 40 minutes.

"	1,200	"	"	20	"	"	"	"
"	1,800	"	"	18	"	"	"	"
"	2,300	"	"	13	"	"	"	"
"	1,080	"	"	10	"	"	"	"

Rabbit, 2,400 grammes, none absorbed, added 4 cubic centimeters in 40 minutes.

Four cubic centimeters were added to the last injected fluid; nothing abnormal in the peritoneum could be noted at the autopsy. Here the average quantity of a 0.75 per cent. Na.Cl. solution absorbed from the peritoneal cavity in 40 minutes is 17 cubic centimeters, or about 275 drops, or  $4\frac{3}{4}$  drams, over  $\frac{1}{2}$  ounce.

To show what almost every experimenter observes—individual differences, the personal equation—we will select two of Adler's and Melt-

zer's experiments. These experiments, the authors state, were made under similar conditions as others which, as noted, were very different in results.

Experiment 35A. Female rabbit, 1,780 grammes; not starved; ligated right innominate vein; injected 100 cubic centimeters of 0.75 per cent. sodium chloride; killed after forty minutes; collected 93 cubic centimeters; absorbed only 7 cubic centimeters (influence of ligation?).

Experiment 35B. Female rabbit, 1,800 grammes; injected 100 cubic centimeters of 0.75 per cent. sodium chloride; killed after forty minutes; recovered 112 cubic centimeters. Nothing abnormal; recovered 12 cubic centimeters more than injected.

Here are two rabbits almost the same size injected with the same quantity of fluid and continuing over the same time, 40 minutes, but the results are very different. These differences rest on yet unknown conditions. Just exactly as in incipient peritonitis, we cannot prognose the capacity of individual resistance. J. H. Hamberger worked carefully on isotonic fluids and hypotonic fluids to derive some knowledge as regards their influence on peritoneal absorption. Hamberger says that a 0.75 per cent. or a 0.92 per cent. Na.Cl. solution is isotonic with the serum of a rabbit and that a 0.3 per cent. and 0.6 per cent. Na.Cl. solution is hypotonic. The difference in peritoneal absorption between isotonic and hypotonic salt solutions is a recognizable factor. We select two experiments from Adler and Meltzer which will illustrate the matter very well.

Experiment 51. Rabbit, 960 grammes; starved 20 hours; injected 97 cubic centimeters of a 0.9 per cent. solution; after forty minutes rabbit put into deep ether narcosis; abdomen opened, all the fluid collected; recovered 75 cubic centimeters; absorbed 22 cubic centimeters; the abdominal incision sutured carefully; injected 99 cubic centimeters of a 0.3 per cent. sodium chloride; after forty minutes rabbit killed; recovered 30 cubic centimeters; absorbed 69 cubic centimeters.

Experiment 52. Rabbit, 1,200 grammes; starved twenty-four hours; injected 99 cubic centimeters of 0.92 per cent. sodium chloride; etherized; after forty minutes abdomen opened and fluid collected; recovered 91 cubic centimeters; absorbed 8 cubic centimeters. Abdomen closed; injected 100 cubic centimeters of 0.3 per cent. sodium chloride; after forty minutes rabbit killed; collected 77 cubic centimeters; absorbed 22 cubic centimeters.

The rabbit in experiment 51 showed a readiness for both isotonic solution (absorbed 22 cubic centimeters) and hypotonic solution (absorbed 69 cubic centimeters), while in the second both the isotonic solution (absorbed 8 cubic centimeters) was poorly absorbed as well as the hypotonic solution (absorbed 22 cubic centimeters). The experiments

also show that the animal which will absorb isotonic fluid readily will also readily absorb hypotonic fluid. In 3 other experiments it was shown that the rabbit's peritoneum absorbed of a .92 per cent. Na.Cl. solution in 40 minutes 14 cubic centimeters (with ligated right innominate vein), 12 cubic centimeters and with ligation of the right and left innominate veins 7 cubic centimeters. In viewing the experiments it is a striking feature to observe the difference of quantities absorbed in regard to the concentration of the salt solution. The more concentrated salt solution, the less the peritoneum absorbs. The very thin salt solution, e. g., 0.3 per cent., is absorbed in large quantities (69 cubic centimeters) in 40 minutes. In three of Adler's and Meltzer's experiments they seem to show that the lymphatics are without much influence in the peritoneal absorption in hypotonic 0.6 per cent. Na. Cl. solution—for with non-ligated lymphatics the peritoneum absorbed in 40 minutes 39 cubic centimeters; with innominate vein tied 35 cubic centimeters were absorbed, and with both the innominate veins ligated 57 cubic centimeters were absorbed. Experiments show that .6 per cent. Na.Cl. solutions are quite constant in the quantity of the peritoneum absorbed, and solutions above .6 per cent. show considerable variation, while solutions below .6 per cent. show less variation of quantities absorbed.

In summing up conclusions from the foregoing experiments two questions arise, viz.: (a) Do peritoneal fluids pass into the circulation by way of lymphatics? (b) Do peritoneal fluids pass into circulation by way of the blood vessels?

The question cannot be answered categorically, nor can experiment dispose of them without controversy. Vast numbers of experiments are requisite for final decision. For example, a rabbit with ligated lymphatics might absorb a small amount, but that very same rabbit without ligated lymphatics might not absorb much more. More facts must be understood to solve the still unknown individual differences. Experiments must be performed under like conditions and similar concentrated salts. An experiment with ether, or artificial respiration, is not under natural conditions. Again, excluding the lymphatics by ligation may defeat our very purpose by deranging the physiologic balance. For as soon as the thoracic duct is ligated the lymph fluid pressure begins to rise and then the peritoneal fluid may percolate or filtrate through the blood capillaries. The main number of Adler's and Meltzer's experiments shows that ligation of the lymphatics retards peritoneal absorption and diminishes its quantity. With ligated lymphatics the largest quantity of a 0.75 per cent. Na.Cl. solution absorbed in 40 minutes was 22 cubic centimeters. In a 2-pound living rabbit in my experiments 42 cubic centimeters of water were absorbed in 40 minutes with open lymphatics. The ligation of the right thoracic duct percepti-



bly diminished the quantity of peritoneal absorption, but the chief effect occurred through the ligation of the left thoracic duct. Hamberger, in his extensive article, denies any influence exerted by the ligation of the right thoracic duct. The experiments show also that the size of the rabbit is not a criterion of how much fluid his peritoneum will absorb. Small rabbits may absorb much, while large rabbits may absorb little. Sometimes not only no absorption occurs, but more fluid is found in the abdomen than was injected. The variation of absorption from the peritoneal cavity rests on still unknown conditions.

As noted in the discussion of Hamberger's extensive article on peritoneal absorption, he must be given the credit of first systematically observing that fluids disappear from the peritoneum of dead animals. However, Recklinghausen and Ludwig with their pupils record how the diaphragm of dead animals absorbed fluids from 1862 onward.

This announcement of the disappearance of fluids from the general peritoneum of the dead animal by Hamberger is naturally a startling matter, for it menaces the correctness of many pet theories. Now, what shall be the answer to the fact that fluids disappear from the general peritoneal cavity? Is living vital force requisite for peritoneal absorption, or is it in the dead a different process than it is in the living? Is the diaphragm the chief or exclusive locality of absorption? Does the lymph continue to flow through the thoracic duct after death? The above questions cover the scope of the field to investigate. I have found the dead human diaphragm absorb sparingly particles of Berlin blue from fluid as long as 72 hours after death. But after the first 24 hours the diaphragmatic absorption is very scant and questionable. If, as Heidenhain claims, the lymph flows on through the thoracic duct after death, it would appear that the absorption after death belongs to the field of the lymphatics. Hamberger, however, denies the influence of the thoracic duct in peritoneal absorption, yet he supports his assertion on the ground of very limited experiments. Adler and Meltzer performed some experiments to test the peritoneal absorption on the dead rabbits and found:

- (a) Absorbed 30 cubic centimeters in 40 minutes.
- (b) Absorbed 80 cubic centimeters in  $1\frac{1}{2}$  hours.
- (c) Absorbed 90 cubic centimeters in  $4\frac{1}{4}$  hours.

These are in accord with my own experiments.

Here time showed an essential element, as the quantity absorbed increased with time and the absorption in the dead was equal to that of life. In two experiments they removed the viscera (stomach and intestines) and in  $2\frac{1}{2}$  hours after injecting 100 cubic centimeters and closing the abdomen it was found that one had absorbed 16 cubic centimeters and the other 20. But this mutilating process is open to so many

objections it destroys structural and physiologic integrity so that conclusions may not stand the test of time.

In one rabbit, whose right innominate vein was ligated with a bow-knot, absorbed in  $2\frac{3}{4}$  hours 8 cubic centimeters; with the ligature untied the same rabbit absorbed in  $2\frac{3}{4}$  hours 30 cubic centimeters, and the same rabbit absorbed in the next  $2\frac{3}{4}$  hours 50 cubic centimeters. So that the first  $2\frac{3}{4}$  hours, with the right innominate vein ligated, 8 cubic centimeters were absorbed, while the next  $5\frac{1}{2}$  hours with the vein ligated it absorbed 80 cubic centimeters. These experiments show that when the innominate veins were ligated there was greater absorption during life than while dead, and second, that absorption in dead rabbits without ligation of the innominate vein is greater than in the living if allowed to continue a few hours. It is a feature to note that experimenters observe imbibed or oedematous sub-endothelial tissue in the peritoneal absorption of dead rabbits, but not in living ones, yet in some of my own experiments I did notice marked "imbibed" or oedematous sub-endothelial tissue.

Starling and Tubby performed some experiments in the physiologic laboratory of Guy's Hospital in 1894 on the absorption from and secretion into the serous cavities and reported them in the *Journal of Physiology*, Vol. 16 (1894). They are worthy of careful consideration. The questions which they attempted to decide were, which is the path of fluid absorption from the serous cavity, viz.: (a) stomata, (b) the lymphatics, or (c) the blood vessels lying in the serous membrane. It is noted that the experiments of Prochaska, Magendie and others which were instituted to decide the path of absorption proved that the fluids reached the blood by passing through the blood vessel walls. Heidenhain objected that Magendie's experiments were not accurate, and hence not reliable. Ascher, working under Kuhne, confirmed Magendie's labors. Ascher found that a drop of a solution of K.I. dropped on a wound was directly absorbed into the blood with passing through the lymph channels. Magendie found that by injecting poisonous solutions into the pleural cavity that death occurred earlier if the blood volume was diminished and later increased by injecting of fluid into the circulation. This pointed to the dependence on the circulation and direct absorption by the blood vessel walls. Ludwig's pupil, Dybkowsky, noted two methods of absorption, viz.: first, fluids containing suspended particles were pumped through the stomata by the respiratory motion, and the second was an interchange between the blood in the blood vessel and the fluid in the serous cavity, i.e., by diffusion and filtrations. But in spite of Magendie's and many other experiments, we must view with care the experiments of Recklinghausen where he observed the passage of milk through the diaphragmatic stomata. Physiologists are inclined to regard the rapid

absorption of fluid holding in suspension finely divided insoluble particles as due to the apt arrangements of absorbents or lymphatics apparatus with which the diaphragm is provided. The method which Starling and Tubby employed was to anesthetize a dog, place a canula in his thoracic duct and a canula in the urethra and then to inject the fluid into the peritoneal cavity. By this method they attempt to decide whether the fluid injected into the peritoneal cavity would appear in the canula of the thoracic duct or in the one in the urethra. When the experiment was over the dog was killed by blowing air into his veins.

I will quote experiment No. 11 from Starling and Tubby to illustrate methods and conclusions:

"Bitch, about 5 kilos. Canula in thoracic duct and canula in bladder (symphysis split to receive it).

TIME.	LYMPH COLLECTED.	URINE.
From 2.40 to 2.50	2 c.c.m., clear reddish.....	3 c.c.m. yellow, clear.
" 2.50 to 2.55	40 c.c.m. of one per cent. indigo.	Carminc in warm normal saline solution was allowed to flow into the peritoneal cavity
" 2.50 to 3.00	2 c.c.m. clear and almost colorless.....	1.5 c.c.m. blue at 2.57.
" 3.00 to 3.10	1.8 c.c.m. clear and colorless..	1.6 c.c.m. deep blue.
" 3.10 to 3.30	3.4 c.c.m. clear, bluish tinge toward end.....	4.6 c.c.m. very dark blue.
" 3.30 to 4.05	10 c c.m. light blue.....	6.4 c.c.m. some last very dark blue.
" 4.05 to 5.05	7 c.c.m. same color as last specimen.....	3.5 c.c.m. very dark blue.

At 3.50 blood was drawn from femoral artery and centrifuged. The serum was bluish, about the same tint as the lymph between 3.30 and 4.05. Dog killed at 5.15; 15 c.c.m. of blue fluid (containing proteids) recovered from peritoneal cavity. Bile, bright green. No staining of the mesenteric glands. A gland in the anterior mesenterium stained blue."

It may be noted that this dog's peritoneum absorbed 25 cubic centimeters in about  $2\frac{1}{2}$  hours. The dog was anesthetized with morphia and A.C.E. mixture, which may account for the slow absorption. In my experiments I have seen a 2-pound rabbit absorb 40 cubic centimeters from the peritoneal cavity in 40 minutes without anesthetic. In this  $2\frac{1}{2}$  hours 15.7 cubic centimeters of lymph collected and 20.6 cubic centimeters of urine. The blue colors appeared in the urine 7 minutes after injecting the peritoneal. The blue color appeared in the lymph about 20 minutes after the injection. In some of my own experiments I noticed the blue color in the urine 7 to 8 minutes after injection. Starling and Tubby have assumed that what is true of experiments in the pleural cavities is also true for the peritoneum. They have only given one experiment, the above, for the peritoneal cavity. From the experiments above quoted they make the following sweeping conclusions:



"One, experiments show that coloring matters in solution placed in the serum cavities are absorbed directly and rapidly by the blood vessels, and this is accompanied by an interchange between the fluid in the cavities and the blood in the vessels."

It appears to the writer that the above experiments are insufficient to bear out such conclusions.

Heidenhain, with his pupil, Orlow, does not agree with Cohnstein that Na.Cl. solution is isotonic. The first two authors assert that 1 per cent. is isotonic, while Cohnstein claims that 6 per cent. is isotonic. Orlow came to his conclusions that the blood vessels were the means of absorbing the fluid from the peritoneum because, when the peritoneum absorbed considerable quantities of isotonic fluid (serum) 1 per cent. Na.Cl. solution, the lymph stream in the thoracic duct did not distend the duct. This does not seem to me sufficient proof that the blood vessels are the chief absorbents, for the fluid in the peritoneum may have passed into the vast interstitial lymph spaces whose intercommunicating channels permit the accumulation of considerable quantities of fluid. Besides, the peritoneal fluids could first pass into the interstitial spaces and later into the blood vessels.

Cohnstein asserts that if the blood capillaries are the means of absorption from the peritoneum of isotonic Na.Cl. solution there must be a gradual thinning of the blood, which, he says, is never the case. Heidenhain holds that there is a thinning of the blood where peritoneal fluids are absorbed, and attempts to prove it by experiments. We take from Heidenhain's article two tables of experiments to show the dried contents of the blood at the beginning and end of the experiments in both peritoneal absorption and intra-venous injection. The dried quantity of blood per fluid volume will indicate what kind of experiment thins the blood.

1. We have as peritoneal absorption of fluids—

I. Time, 2h. 37m.; quantity per kilo, 10 c. c. m. Na.Cl. 0.6 per cent.; dried contents of serum of experiment at beginning, 7.99 (1) per cent.; at end, 7.54 per cent.; difference, 0.45 per cent.

II. Time, 3h.; quantity per kilo, 14 c. c. m Na.Cl. 5 per cent.; dried contents of serum of experiment at beginning, 8.15 per cent. (2); at end, 7.12 per cent.; difference, 1.03 per cent.

III. Time, 3h.; quantity per kilo, 10.6 c. c. m. Na.Cl. 0.1 per cent.; dried contents of serum of experiment at beginning, 7.46 per cent. (3); at end, 7.21 per cent.; difference, 0.25 per cent.

2. We have as intra-venous infusion—

IV. Time, 3h. 2m.; quantity per kilo, 9.7 c. c. m. Na.Cl. 1 per cent.; dried contents of serum of experiment at beginning, 6.72 per cent. (4); at end, 6.48 per cent.; difference, 0.24 per cent.

V. Time, 2h. 12m.; quantity per kilo, 10 c. c. m Na.Cl. 1 per cent.; dried contents of serum of experiment at beginning, 8.02 per cent.(5); at end, 7.49 per cent.; difference, 0.52 per cent.

(1) Tested 15 minutes after infusion in abdomen, (2) 10 inches and (3) immediately before its infusion. (4) and (4) immediately before the infusions.

In the above experiments during the same time and with equal quantities of fluid injected into the abdominal cavity or in the veins, the blood serum changed the relation of its watery and dried contents to a considerable degree.

The above experiments were performed on dogs as the following:

1. Dog, weight, 5.6 kilo; quantity injected in abdomen, 125 c. c. m. 0.63 per cent. Na.Cl. solution.

2. Dog, weight, 7.5 kilo; quantity injected in abdomen, 125 c. c. m. 0.63 per cent. Na.Cl. solution.

3. Dog, weight, 7.5 kilo; quantity injected in abdomen, 240 c. c. m. 0.1 per cent. Na.Cl. solution.

4. Dog, weight, 11. kilo; quantity injected into vein, 106 c. c. m. 0.1 per cent. Na Cl. solution.

5. Dog, weight, 10. kilo; quantity injected into vein, 100 c. c. m. 1. per cent. Na.Cl. solution.

The blood serum changes its watery contents only within narrow bounds whether the injection be made into the abdominal cavity or the cavity of the veins.

According to the results of the above experiments from Heidenhain, the difficulty of discriminating between absorption from the abdomen or by way of the lymph paths or blood vessels is increased. Heidenhain's experiments contradict Cohnstein's assertion that the blood is not thinned by absorption from the abdominal cavity. Cohnstein asserts that if the blood capillaries absorbed the peritoneal fluid the secretion of urine must increase, which is never the case. To this proposition Heidenhain partially agrees, for he says that if isotonic Na.Cl. solution be injected into the veins as slowly as the peritoneum absorbs fluids, that the secretion of urine shows no essential increase. But Heidenhain questions the measuring experiments of urine conducted by Cohnstein. Cohnstein asserts that the massage of the abdomen or elevation of the posterior portion of the body, or in any method which increases the intra-abdominal pressure during peritoneal absorption will increase the stream of lymph from the thoracic duct. But this appears to be no proof of either absorption from the peritoneum by lymph or blood vessels, for massage of the abdomen, elevation of the posterior portion of the body or any method which increases intra-abdominal pressure will increase the flow of lymph from the thoracic duct. Perhaps the reason

lies in the fact that the centrum tendineum is endowed with the highest power of absorption of any portion of the peritoneum, and any fluids which approach it will be quickly absorbed.

Heidenhain demonstrates this in an experiment on a dog.

The lymph quantity flowing from the thoracic duct in 10 minutes :

1. By a horizontal position of dog, 2.4 c. c. m.
2. By the elevating the posterior portion of dog, 3.3 c. c. m.
3. By horizontal position of dog, 2.4 c. c. m.

Again, Cohnstein claims that if the absorption from the peritoneal cavity occurs through the lymphatics, the flow of lymph from the thoracic duct must increase during the experiment. If a dog be bound and a canula be placed in the thoracic duct, the flow of lymph from the canula will continually decrease. Now if the abdominal infusion makes the flow of lymph from the canula constant, it speaks in favor of lymphatic absorption. Cohnstein claims that this is just what his experiments demonstrated. It is well known that the lymph flows slowly through the thoracic duct and any manipulation or trauma, as, for example, introducing the canula in the thoracic duct, will retard the flow for awhile. But after the consequent lymph congestion is relieved and the stream begins to assume a regular flow, it will go steadily on. If the animal's temperature falls, the lymph stream from the thoracic duct will gradually decrease. But Heidenhain claims from his experiments that if the dog's temperature is kept up by artificial heat the lymph flow from the thoracic duct does not decrease.

Zawilski, in 1876, in the physiologic institute at Leipsic, made some observations over the lymph stream and during the digestion of fat, and he found in (1) examination that the flow of lymph from the thoracic duct first increased and then became constant; (2) it increased and then decreased; (3) it continually decreased; in (4) it was constant for 1 hour, then during 2 hours again constant, becoming a little less than the first, and in (5) during  $2\frac{1}{2}$  hours constant and then decreasing. These experiments show that increase or decrease of flow of lymph through the thoracic duct is a slow matter. What is the relation between the amount of fluid absorbed from the abdomen and the amount of lymph collected from the thoracic duct? Heidenhain performed three experiments which show some of these relations.

- |       |   |  |
|-------|---|--|
| In 1. | { | 55 c. c. m. were absorbed from the abdomen, and                |
|       | { | 30.75 c. c. m. of lymph were collected from the thoracic duct. |
| In 2. | { | 104 c. c. m. were absorbed from the abdomen, while             |
|       | { | 31 c. c. m. of lymph were collected from the thoracic duct.    |
| In 3. | { | 80 c. c. m. were absorbed from the abdomen, while              |
|       | { | 42 c. c. m. of lymph were collected from the thoracic duct.    |



Here 239 c. c. m. were absorbed from the abdomen, while 103 c. c. m. of lymph were collected from the thoracic duct.

(The solution absorbed was 0.6 per cent. to 1 per cent. Na.Cl.)

At a glance one can see that no average can be struck in the cases, for the absorption of fluids from the abdomen is two or three times as much as the collected lymph. I found in my own experiments that the abdomen absorbed under apparently similar conditions very different amounts of fluids. If the thoracic duct does not deliver more than  $\frac{1}{2}$  to  $\frac{1}{3}$  of the amount of fluid injected into the abdominal cavity, we must look for the fluid in some other place, in (a) the interstitial lymph spaces, (b) in oedema of the sub-endothelial tissue or (c) in the blood vessels. I have never observed but slight traces of oedema in the experiments on the living.

Cohnstein found that the solid contents of the lymph decreased about 0.3 per cent. after the injection into the peritoneal cavity of an isotonic Na.Cl. solution. He does not, however, state where the water comes from that dilutes the lymph. Heidenhain combats this idea by saying that if one injects into the abdominal cavity what will disappear in 2 to 3 hours, the lymph will thin just the same as if one injects a like quantity into the vein. Here Cohnstein attributes to lymphatic absorption what Heidenhain attributes to absorption by blood vessels. To prove his assertion that fluid injected into the vein thins the lymph just the same as fluid absorbed from the peritoneal cavity, Heidenhain took a dog weighing 10 kilos and injected into his jugular vein 100 c.c.m. in  $2\frac{1}{4}$  hours. In the beginning the dried content of the lymph was 6.18 per cent. and at the end 5.68 per cent., i. e., the lymph thinned 0.5 per cent., which is a little more than Cohnstein's 0.3 per cent. Cohnstein claims that if the blood vessels absorbed the abdominal fluid that the blood should become thinned. This he claims does not happen to any considerable extent, while Heidenhain claims that it does thin, but slowly.

Cohnstein expected from absorption of the blood capillaries that the secretion of urine would be essentially increased, but it did not increase the secretion of urine. But the urinal secretion did not increase when the infusion was injected into the blood if it were done slowly or during the same time it required for peritoneal absorption.

Cohnstein drew a conclusion for the lymphatic absorption from the effect of the lymphatic increased lymph stream. But massage of the dog's abdomen, or elevation of the dog's hind legs, will do the same thing. Increase of the lymph stream cannot be counted on as indicating peritoneal absorption, for massage or change of position will effect the same.

Cohnstein held that because the lymph stream does not lessen dur-

ing the whole experiment that it indicates the lymphatics are absorbing instead of the blood capillaries, for during experiments the lymph stream gradually lessens. But the increase of lymph, if it occur, is not in reasonable relations to the quantity of peritoneal absorption. Also by direct infusion into the blood occurs a similar increase during a similar length of time.

During the experiments the lymph becomes a trifling degree thinner.

Heidenhain sums up his own and Orlow's conclusions finally by saying that he is of the opinion that the essential peritoneal absorption is performed by the blood capillaries of the peritoneum. He and Orlow both admit that Recklinghausen's lymph tracts may also absorb some fluid.

Hamberger of Utrecht, Holland, came to similar conclusions. Heidenhain and Hamberger agree that osmose is not the force by which isotonic solutions can be absorbed. The force must lie in the walls of the abdominal cavity. So far Heidenhain and Hamberger agree, but further than this they separate in opinion. Heidenhain holds to having "vital" forces. But all of us who have experimented know that the dead peritoneum will absorb similar to the living, at least for many hours after death.

Heidenhain, though he confessed he was astonished at the fact of dead animals absorbing fluids, yet proclaims that the absorption in the dead and living is entirely different, but gives no data to convince us of his opinion—only the promise of a future article.

The labors of Heidenhain and Orlow, his pupil, in regard to peritoneal absorption may be here discussed, as they essentially agree. The following is a short resume of some of their views:

1. The lymphatics are not the essentials in absorbing fluids from the peritoneal cavity, but it belongs to the blood vessels.

2. A series of facts, observed during absorption, may be represented by the action between blood vessels and abdominal contents.

3. With the osmotic appearances in absorption arise other functions for which a physical process cannot fully answer. They indicate forces which take their origin in the abdominal walls. Doubtless they mean "vital forces."

Cohnstein asserts that what is not done by osmosis in absorption is performed by the blood vessels. This induces Heidenhain to defend himself and his pupil, Orlow, against Cohnstein's conclusions. First, Heidenhain claims that Cohnstein does not practice osmosis as is known to the physiologists today, either physically or experimentally. Heidenhain then lays down the following as the present teaching of osmosis: "If a watery solution of unlike endosmotic tension be placed on either

side of a (diffusion) membrane, the fluid of less tension will pass to that of the greater tension on the other side."

Cohnstein asserts that the capillaries of the peritoneal membrane will absorb fluids like the capillaries of other similar membranes, but the effective power is the osmose. The difference of opinion here between Heidenhain and Orlow and Cohnstein is in regard to the tension or density of fluid (whether hypotonic or hypertonic). Of course, if we have hypotonic or hypertonic fluids in the peritoneal cavity, the fluid of the blood capillaries must pass to such solutions if we follow the law of osmosis.

The question of peritoneal absorption is not only of physiologic worth but of great pathologic interest; however, the pathologic process must depend for its interpretation on the function or physiology. We may note that in cardiac disturbances or hepatic lesions fluids collect in the peritoneal cavity, sometimes slowly, sometimes rapidly, and disappear in the same irregular manner. I once had a patient in whom per-ovarian and ovarian cystic tumors would collect fluid for a long time and then suddenly rupture, whence the abdomen would at once become flat and the urine would be enormously increased for several days. This form of rupture occurred three times in one year. Pouring water in dog's peritoneum and closing the abdomen with sutures produced similar effects, as witnessed by frequent urination for 24 to 48 hours.

Ruptured tubal pregnancies may pour large quantities of blood into the peritoneum, but it may become absorbed. Not only blood, but milk, fluids containing solids, oil, etc., will become absorbed. In fact, the peritoneum is a good cavity to inject a physiologic salt solution in cases of excessive bleeding, for I have seen a female dog of 7 pounds absorb 95 cubic centimeters (about 3 ounces) in 30 minutes. Perhaps the peritoneal cavity will absorb fluid quicker than the cellular tissue under the skin. What is the path of peritoneal absorption, and by what power is it accomplished? Investigators have been trying to determine the answer to these questions for over a generation. Is it osmosis, stomata, filtration, imbibition or physiologic power residing in the endothelial cell? Perhaps no one of these will entirely answer. It is remarkable how much fluid the peritoneal cavity will dispose of in a short time. In my experiments the peritoneal cavity disposed of quantities of fluid as high as 10 per cent. of body weight in 30 minutes. Most of the experiments were for thirty minutes, during which time one could observe a dog of about 7 pounds absorb 3 ounces from his peritoneal cavity, or about 10 per cent. of body weight. The intensity of peritoneal absorption depends on the intra-abdominal pressure. If one injects into the peritoneal cavity large quantities of fluids the peritoneal absorption will be relatively more rapid than when small quantities are injected.



Dr. Georg Wegner indicates four possible ways by which the fluids may become absorbed from the peritoneal cavity:

1. By diffusion or osmosis.
2. By filtration, i.e., by intra-abdominal pressure.
3. The endothelial cells and the wandering cells.
4. Through the diaphragm, i.e., the path demonstrated by Von Recklinghausen in 1861.

In 1880 Dr. Nikolsky wrote a thesis on the subject of defibrinated blood in the abdominal cavity. He notes, according to Dr. Orlow's translation from the Russian, that injecting 300 c.c.m. in the abdomen, 5 hours later no blood was absorbed, but at the end of 24 hours 160 c.c.m. were absorbed. He also found by microscopic examination that the lymphatics of the diaphragm were filled with the injected blood corpuscles. This irregular absorption of fluids by the peritoneum noted by Nikolsky is the common observation of all experimenters, e.g., under similar conditions one dog of 15 pounds absorbed only 8 c.c.m. in 30 minutes, while one of 7 pounds absorbed 95 c.c.m. in our experiments. Cordua, Prof. Ponfick's pupil, noted that three days after he had injected defibrinated blood into the peritoneal cavity he found only half absorbed, and after five days the abdomen was entirely empty. In my experiments the intensity of peritoneal absorption often astonished me, but the irregularity of the absorption of the peritoneum in time and quantity is remarkable.

Dr. W. N. Orlow, working in the laboratory of Prof. R. Heidenhain, in Breslau, Germany, conducted some excellent experiments in regard to peritoneal absorption on dogs in 1894. His general plan was to weigh the dog, inject morphine (2 to 100) under the skin, a cubic centimeter for every kilo of body weight. In 15 to 20 minutes the dog was bound on a table and narcotized with a mixture of ether and chloroform after which the ductus thoracicus was laid bare and a canula introduced to collect the lymph. Fluid at 39 degrees to 40 degrees Centigrade was injected into the abdominal cavity. After 3 to 5 hours the dog was killed, the peritoneal fluid collected and the percentage of Na.Cl. determined for the fluid found in the abdomen as well as that injected. The fluid injected was dog's serum which was placed on ice, and just before injecting it was warmed to 39 degrees—40 degrees C.

In the annexed table of experiments the blood serum of the dog was injected at 39 to 40 Centigrade, and with a like endosmotic force with the dog's blood plasma. The serum was collected from dogs and laid aside until used, when it was warmed to 39 to 40 degrees. Nos. 1 and 2, which had a duration of experiment of 7 and 8 hours, showed peritoneal extravasation. The other 5 are not noted. Nos. 3, 4 and 6, on which the experiment lasted 7 hours, were continually under chloroform.

No. of Experiment.	Body Weight.	No. c. c. m. of blood serum injected.	Duration of experiment.	No. of c. c. m. of blood serum found in abdomen.	Organized substance in the injected fluid. Per cent.	Organized substance in the re-col-lected fluid. Per cent.	Organized substance in blood serum of animal. Per cent.	Inorganic substance in blood serum absorbed.	Per cent. of fluid injected in reference to body weight.	Per cent. of fluid absorbed in reference to body weight.	Remarks.
No. 1..	10.000	246	2	110	....	....	....	136	2.46	1.36	{ Without chloroform narcosis. morphine narcosis.
No. 2..	17.500	175	7	0	....	....	....	175	1.	1.	{ Without chloroform narcosis. morphine narcosis.
No. 3..	10.400	200	7	150	7.270	7.138	7.230	50	1.82	0.48	{ With chloroform narcosis. Without morphine narcosis.
No. 4..	10.600	205	7	130	....	....	....	75	2.05	0.75	{ With chloroform narcosis. Without morphine narcosis.
No. 5..	5.200	175	6½	108	6.194	6.546	6.602	67	3.36	1.3	{ Without chloroform narcosis.
No. 6..	11.100	321	7	242	6.690	7.044	7.944	79	2.08	0.71	{ Without chloroform narcosis.
No. 7..	5.660	112	7½	156	8.676	6.930	6.648	44	2.	....	{ This blood serum was in vacuo concentrated.

In No. 4, the rate of outflow of lymph before the injection was 5.6 c.c.m. in 10 minutes. After the injection, 3.2 c.c.m. in 10 minutes; in No. 4, before the injection, the rate of lymph flow was  $5\frac{1}{2}$  to 6 c.c.m. in 10 minutes, while after the injection in the peritoneal injection,  $3\frac{1}{2}$  to 4 c.c.m. in 10 minutes. The three elements which could be objected to in Orlow's experiments were: (a) morphine injections, (b) chloroform narcosis, and (c) binding the animal on a bed during the experiment. The most natural method would be to inject the blood serum into the abdominal cavity, after which allow the dog perfect freedom. Observe that the rate of lymph flow decreased after the blood serum was injected into the peritoneal cavity. In No. 7, it will be noted that not only there was no peritoneal absorption, but the injected fluid was increased by a transudation from either the interstitial lymph spaces or the blood capillaries of the peritoneum. Observe in the above 7 experiments that the omentum absorbed in reference to body weight was 1.36 per cent., and the lowest was 0.48 per cent.

In some of my experiments the per cent. of absorption in regard to the body weight was as high as 3 per cent., and some sank as low as .25 per cent. Bodily motion enhances peritoneal absorption and the rate of lymph flow. Morphine and chloroform, in so far as they checked bodily action, would, in proportion, check peritoneal absorption and lymph flow. Now, when blood serum of like composition as the blood serum of the experimented animal, and having the same endosmotic power, is injected in the peritoneum and yet a lively peritoneal absorption of the fluid occurs, it cannot be attributed to osmosis. If the fluid was absorbed by way of the lymphatics, one would think that the rate of lymph flow from the thoracic duct would increase after the injection, but in Nos. 4 and 6 it actually became less after injection. Yet we must not forget that the fluid might have rapidly disappeared into the interstitial lymph spaces, and that some time (hours) later the lymphatic duct would have an abnormally higher rate of flow. From the above rather few experiments, Orlow claims that the blood vessels of the peritoneum must have the essential function of absorption. As the sentence is significant, we will quote it from his own article: "Mithin Muessen es die Bluthbahnen sein, welche die wesentliche Function bei der Resorption haben." The stress must here be placed on "wesentliche," which, doubtless, ordinarily means essential, which does not exclude the lymphatics from the field of absorption. When the process of osmosis does not reasonably explain the passage of fluid from the peritoneal cavity, we must assume some other seat of power, whether that be in the endothelial cell of the peritoneum, of the blood vascular system, of the lymph vascular system, or in the interendothelial structures. Now, in experiment No. 7, of Orlow, the blood serum concen-



trated in vacuo had a higher osmotic power than the blood serum of the capillaries; hence the thinner capillary or lymphatic fluids must necessarily flow toward the thicker fluid contained in the peritoneal cavity, so that instead of the 112 c. c. m. injected becoming less, it becomes increased by 44 c. c. m., and at the end of the experiment 156 c. c. m. were found.

## CHAPTER IX.

### THE TECHNIQUE:

#### OR METHODS OF PREPARING SPECIMENS OF THE PERITONEUM FOR MICROSCOPICAL EXAMINATION.

"We do well what we do automatically."

The endothelia of the peritoneum in a fresh state is so homogeneous and transparent that time is wasted by examining it without reagents to develop to the eye the forms and structure of its various elements. One can note, however, in the fresh state the dim outline of the endothelial plates. The homogeneity of the protoplasm of the endothelial plates in the peritoneum makes the specimens in the fresh state poor in demonstrating facts. For this reason various liquid reagents have been employed to demonstrate the outlines of the endothelial plates, such as Ag.  $\text{NO}_3$ , osmic acid, tannin, potassium bichromatic, eosin, hot water, gold chloride, logwood, etc.

There are two kinds of reagents required to demonstrate the structure and outline elements of the peritoneal membrane on its free surface, a fixation and a coloring reagent. One kind of reagent fixes immovably the endothelial plates and structures in the normal relations. The other kind so colors the structures and outlines of the endothelial plates that they become visible even to the naked eye or the lens and can be accurately studied by the aid of the microscope. The fixation reagents are chiefly bichromate of potassium, Muller's fluid, osmic acid, tannin, formaline and alcohol. The chief coloring reagents for structure and outline of the peritoneal endothelia are Ag.  $\text{NO}_3$ , logwood, eosin and methylin blue.

To color the structures on the free surface of the peritoneal surface we used, chiefly, two reagents, viz.: Ag.  $\text{NO}_3$  and logwood. The silver colors the interendothelial space and marks a portion of the plate surface brownish, while the logwood colors the nucleus of the endothelial plate.

To bring into view the endothelial plates and interendothelial spaces of the free peritoneal surface, a freshly killed rabbit's or other animal's abdomen is opened with as little trauma as possible. On a portion of

the omentum a  $\frac{1}{4}$  per cent. solution of  $\text{Ag. NO}_3$  is gently poured, and allowed to remain for two minutes, after which it is removed with a pair of forceps and a sharp pair of scissors and placed in a capsule of water in the sunlight (daylight) from a few minutes to two hours. Snip off a small transparent bit of the omentum in the capsule, float it on a slide, place a drop of glycerine on a cover-glass and apply it to the specimen. The microscope will then demonstrate the brown endothelial plates and the dark interendothelial space and their associated structures. Should one want to use other reagents on the endothelial membrane than the  $\text{Ag. NO}_3$  or develop a second silver stain, the first silver stain must be made very light, say,  $\frac{1}{4}$  per cent.  $\text{Ag. NO}_3$  for a quarter of a minute and the further action of the silver checked by placing the specimen in a 1 per cent.  $\text{Na.Cl.}$  solution.

To bring the nucleus of the endothelial plate into view, place the specimen in logwood for  $\frac{1}{2}$  to 2 minutes, after which wash it in water and mount in a drop of glycerine. Under the silver method of staining to secure perfect specimens, we must have fresh, non-traumatized endothelial membrane. It should not be smeared with blood. The medium of treatment should be distilled water and sunlight.

In the microscope (low power) we observe dark lines which bound clear spaces, and in the clear spaces, which are the endothelial plates, we observe oval or round clearly defined nuclei. The endothelial plate shows itself brownish and granular toward the circumference, but lighter toward the center, said by some to be due to the centrally elevated portion of the cell, and hence the  $\text{Ag. NO}_3$  solution flows chiefly toward the edges.

The peritoneal endothelia well prepared by  $\text{Ag. NO}_3$   $\frac{1}{4}$  per cent. for five minutes, logwood one minute and sunlight for two days, shows under 500 power a complicated structure. The endothelial plate appears dark brown or yellow granular, and possesses generally towards its center an oval or round clearly defined vesicular nucleus with occasionally one or more nucleoli. The substance of the plate with a still higher power represents a reticulated granular surface. If the endothelial plate be exposed to strong sunlight too long it loses its transparency and distinctness. By careful observation of the dark interendothelial lines it will be easily noted that they are composed of two parallel lines and the space between them is crossed by transverse anastomotic protoplasmic processes which bind the endothelial cells into colonies. The transverse protoplasmic processes of the interendothelial space are very fine and thin at the surface, but thicker and broader as they descend from the surface. The darkening of the parts in the interendothelial space is due to the precipitation of a fine layer of albuminous fluid which bathes them. Also in the interendothelial space of the common



junction of several endothelial plates are noted apertures or structures surrounded by granular protoplasmic polyhedral cells which stain highly brown by the Ag. NO<sub>3</sub> solution. These structures or apertures are known as stomata vera. They are vertical lymph channels which directly connect the peritoneal cavity with the lymphatics of the sub-peritoneal tissue. On the linear interendothelial cleft there are situated structures, dots, rings and clumps of matter which are known as stomata spuria. The stomata spuria and vera are designated by some stomata and stigmata, especially by Prof. Arnold, of Heidelberg.

The stomata spuria are connective tissue corpuscles or leucocytes projecting upward between the cells. Some claim the stomata spuria are artificial products. The peritoneal endothelia should be studied in its various stages from a few minutes to a month after the effect of the reagents, Ag.NO<sub>3</sub> and logwood. The beginner should distinguish between the cover-plate, i.e., the superior metamorphized protoplasm and the inferior protoplasmic nucleated portion, the real part of the endothelial cells. The chief type of all peritoneal stomata are those situated on the frog's or turtle's (amphibian) lymph sac. This sac is known as the cisterna lymphatica magna. The wall of the sac is covered on the abdominal side by peritoneal endothelia and on the cisternal side by lymphvascular endothelia. Both sides present the chief type of stomata vera and are prepared in the same way as peritoneal endothelia. The Ag. NO<sub>3</sub> solution stains the granular, protoplasmic polyhedral nucleated cells of the stomata vera on the lymph sac an intense brown color. The cells lining the stomata vera are the lymph sac, apparently very fine, 2 to 6 or 8.

The silver nitrate solution produces an intense brown stain on certain endothelial cells of the various portions of the peritoneum known as germinal endothelia. These germinating endothelia are found more especially on the gastro-splenic omentum. The germinating endothelia may be arranged in the form of an ornamental mosaic or nodules elevated above the common endothelia or irregular patches which possess much vascularity. We discard the term interendothelial cement substance and replace it by interendothelial space. By intensely staining the peritoneal endothelia with Ag.NO<sub>3</sub> solution, very frequently more or less of the subperitoneal lymphatic system may be observed on account of the transparency of the peritoneal endothelial plates. But a better or more certain method should be employed.

The method to demonstrate the stomata vera on the diaphragm where they are most numerous and certain to be found is to open the abdomen of a recently killed rabbit (or other small animal), pour a  $\frac{1}{2}$  per cent. solution of Ag. NO<sub>3</sub> over the abdominal surface of the diaphragm and allow it to remain on it for five to eight minutes. After

which cut the diaphragm out with a sharp pair of scissors with as little trauma as possible and place it in a dish of distilled water in the sunlight for a few minutes to a few hours, after which preserve it in a 5 per cent. solution of formaline, a 75 per cent. solution of alcohol or Muller's fluid (which last should be renewed in thirty-six hours). From a few minutes after the diaphragm is removed from the animal and placed in the distilled water to a month later one can examine certain portions of the centrum tendineum snipped off by sharp scissors. No preparation is required to mount the specimens except a slide and a drop of glycerine and a cover-glass. If one wishes to observe the lymphatics of the centrum tendineum all that is necessary is to allow a watery solution of Berlin blue to lie in contact with the centrum tendineum (dead or alive) for ten or more minutes. In a mounted specimen of the diaphragm (centrum tendineum) one observes the endothelial membrane covering the radiating bundles of tendons and their intertendinous spaces, stomata vera, i. e., granular protoplasmic polyhedral nucleated cells surrounding an aperture, may be observed at the common junction of several endothelial plates, especial attention is called to the intertendinous space. In the interendothelial membrane only a few stomata vera are seen directly over the tendinous bundles of the diaphragm, but stomata vera, frequently arranged in rows, are clearly seen in the endothelial membrane between tendinous bundles of the centrum tendineum. If a watery solution of Berlin blue had been allowed to remain in with the centrum tendineum for a short time (better) after death the intertendinous spaces will be observed with the Berlin blue in the channels. It must be stated, however, that stomata vera in any portion of the peritoneum are irregular in number and distribution. On the diaphragm they are most numerous and certain of demonstration. The Ag.  $\text{NO}_3$  solution will demonstrate nucleus, but one minute in logwood develops a beautifully clear nucleus. The nuclei of the cells lining the stomata vera can be demonstrated by silver and logwood, but they are uncertain and there may be one or more, as in the peritoneal endothelia. The stomata spuria may be easily demonstrated on the linear endothelial cleft as rings, dots or masses of matter. Objects very similar to the stomata spuria (pseudo-stomata) may be produced by stretching or traumatizing the endothelial membrane of the peritoneum. The stomata spuria may be leucocytes emerging or clamped between the cover-plate, lymph corpuscles or connective tissue cells.

TO DEMONSTRATE THE LYMPHATICS OF THE DIAPHRAGM BY INJECTIONS OF  
BERLIN BLUE.

Inject into the rabbit's abdomen 100 c. c. m. of a watery solution of Berlin blue, with a trachar and a canula. Kill the rabbit in thirty

minutes to 24 hours later. It appears to me to make little difference with starved or non-starved animals. When killed, open the abdomen and chest. Brush both sides of the diaphragm gently with a camel's-hair brush (or a toothpick on which cotton is wound) two to three times and pour on both sides of the diaphragm a  $\frac{1}{2}$  per cent. solution of  $\text{Ag. NO}_3$  for 5 to 10 minutes. Afterwards, carefully, with a pair of sharp scissors cut out the whole diaphragm by following the chest wall in its whole diaphragmatic circumference and, finally, the posterior portion where the tendinous portion is inserted. Place the whole in a vessel of distilled water. By examining the diaphragm as it floats in the water with the naked eye or lens we may observe the Berlin blue deposited in the radiating intertendinous spaces. Mounted microscopic specimen of this injected centrum tendineum shows the lymphatics loaded with Berlin blue. The particles of blue are deposited in all the lymphatic vessels of the diaphragm, especially in the intertendinous spaces with its lateral sinuses and bulgings, in the superficial and deep, straight vessels, in the short, vertical vessels, in short, the Berlin blue will be found in the valved and non-valved lymph capillaries, and in the vast interstitial spaces.

To demonstrate the lymphatics of the omentum, which is very convenient to treat with reagents, open the animal's abdomen, brush the portion of the omentum desired for examination in situ gently two or three times, after which pour on a  $\frac{1}{2}$  per cent. to  $\frac{1}{4}$  per cent. of an  $\text{Ag. NO}_3$  solution and allow it to remain three to five minutes. Carefully cut away the omentum and place it in a vessel of distilled water in the sunlight. Mount small pieces in glycerine. One observes in the specimens successfully prepared (a) valved lymphatic trunks, which, however, are difficult to demonstrate, and in most parts of the omentum, especially below the transverse colon; (b) non-valved lymph capillaries; (c) interstitial spaces; (d) germinal endothelia in the form of mosaics, patches or nodules; (e) vacuolation of cells forming lymph patches. The vacuoles become rapidly covered by cells, which flatten out into endothelial plates. The vacuolation gradually becomes lymph spaces. In the turtle, especially in the breeding season, one can observe the regeneration of the endothelial plates.

#### THE METHOD TO DEMONSTRATE THE NERVES OF THE PERITONEUM.

Take a fresh piece of non-traumatized peritoneum, pour on it  $\frac{1}{4}$  per cent.  $\text{Ag. NO}_3$  for  $\frac{1}{2}$  to 1 minute, after which wash it in distilled water for 2 minutes. Place the specimen in gold chloride 1 part, plus acetic acid 3 parts, plus  $\text{H}_2\text{O}$  996 parts 20 to 30 minutes. Begin at the end of 20 minutes to snip small bits from the specimen in the gold chloride and acetic acid solution and remove them to a solution of 2 to 4 parts



acetic acid in 200 to 400 parts of water. The variation of the action of the gold chloride will in this manner become observed in the specimens. The specimens may be well preserved for nearly or quite a week in the acetic acid 1 part and  $H_2O$  200 parts, but it is better, so far as my experiments are concerned, to place the specimens stained in gold chloride and acetic acid in a solution of 1 part acetic acid and distilled water 400 parts, in strong sunlight for a week, examining the specimens daily by mounting in glycerine. By examining the mounted specimens from the gold staining, we may observe (a) that the delicate silver stain was just sufficient to definitely mark the outlines of the endothelia, which will present landmarks or relations of structures; (b) we note bundles of medullated nerve trunks, on the lateral borders of which may be seen non-medullated nucleated nerves; (c) Remak's bands, possessing a nucleated sheath, may be observed mixed with medullated nerves. (d) Around the small vessels we can note a finely spun, dark network of non-medullated nucleated nerves. (e) The peritoneal nerve network is anastomosis by contact only. (f) The non-medullated nerves appear to end in the granular protoplasm of the cells surrounding the mouth of the stomata vera. (g) As many as half a dozen various kinds of endings of nerves may be noted in the peritoneum. (h) The especially known form of nerve terminals is best found in the cat's mesentery, called the Vater-Pacinian corpuscle. These are visible to the eye as pale or pearly oval bodies of various size. Gold stain presents the most beautiful stain known, but is uncertain and evanescent. Gold stain is generally unreliable after 10 days.

Perhaps the nerves of the peritoneum are most easily and certainly demonstrated on the cisterna lymphatica magna. Snip off a piece of the lymph sac, place it in a solution of gold chloride 1 part, acetic acid 5 parts, and  $H_2O$ . 995 parts for 15 to 40 minutes. At the end of 15 minutes begin to snip off small bits of the specimen and place them in acetic acid 1 part and  $H_2O$ . 400 parts in good sunlight. Mount the specimens in glycerine for examination. The peritoneum over the lymph sac is a typical locality for the examination of the peritoneal nerves, because blood vessels and elastic fibres are very scarce in this region, and hence the nerve fibres are not confused with them. The nerve fibres consist of double contoured nerves, single contour or non-medullated nerves, nerve nuclei and Remak's bands enclosed in a nucleated sheath.

An additional method is to treat the endothelial membrane as above suggested with gold chloride and acetic acid. After the endothelial membrane has become brown in the sunlight the endothelia may be penciled off. It may then be placed for  $\frac{1}{2}$  hour or more in an alkaline solution of carmine, when it is washed and ready to be mounted in

glycerine. This method shows well the network of non-medullated nerves about the vessels. Some authors claim and illustrate medullated nerves ending on the blood vessels, of which a cut is presented on page 175, a figure borrowed from Corlier, Hayercroft and Scofield.

The nerves forming the network around the blood vessels are with successful staining very numerous, in fact, so numerous that doubt may be entertained as to every stained strand being a non-medullated nerve-strand. The nerves terminate about the vessels, as bulbs and network, apparently by contact.

Another simple method to prepare the nerves of the peritoneum for microscopical examination is to take 100 c.c.m. of distilled  $H_2O$ , add 12 drops of  $H.C_6H_3O_2$ . To this mixture add 25 drops of  $\frac{1}{2}$  per cent. of gold chloride and allow the peritoneal specimens to remain in this solution in the sunlight for one day to a week, examining the specimens daily. For this work secure specimens from the wall of the lymph sac and mesogaster of amphibia, from the mesentery and gastro-splenic omentum of the cat or rabbit; especially good specimens may be obtained from the rabbit from the folds of peritoneum which stretch from the diaphragm to the stomach or liver.

To demonstrate the endothelia lining the blood vessels of the peritoneum: The omentum of a rabbit or thin portion of the peritoneum of any animal stained with  $Ag.NO_3$  for ten minutes and placed in distilled  $H_2O$  in good sunlight for 1 to 48 hours generally is sufficient to develop the dark interendothelial lines of the vascular (capillary) endothelia. The stomata vera and spuria may be observed. The specimen mounted in glycerine shows that the fine capillary vessel wall is composed of endothelial plates so arranged, edge to edge, as to form a tube. The capillaries by this method of staining may be noted to form a network in the peritoneum. The blood vessels may be more definitely demonstrated by brushing off the endothelia and afterwards staining with  $Ag.NO_3$ ; mount in glycerine.

Another method is to inject the blood vessels with  $Ag.NO_3$   $\frac{1}{2}$  per cent. solution, after which wash them out with  $H_2O$  distilled; place in the sunlight and mount in glycerine. Still another method is to inject into the vessels carmine or other coloring agents and it will be deposited in the interendothelial space of the vascular endothelia.

To demonstrate the lymphatics of other localities of the peritoneum, as the omentum majus, the pyloric region, the mesentery or the broad ligament, we need to follow the simple directions of gently brushing the free endothelial surface 2 to 4 times with cotton wound on a toothpick and staining the part of the peritoneum in situ so as to avoid dragging (a camel's hair brush is perhaps the more delicate, hence to be preferred). All the trauma, dragging or rough handling should be avoided

or the lymphatics not only collapse, but the endothelia and interendothelial space are disturbed.

The gynecologist may study with profit the vast bed of lymphatics found in the subserous tissue of the (human) ligamenta lata. The lymphatics on the broad ligaments on the interstitial space vary, and though very fine are quite extensive. Near the pylorus there exists a rich system of subperitoneal lymphatics. In the frog the peritoneum covering the stomach is rich in interstitial spaces. When one stains the mesogaster of a frog or turtle, the peritoneal serosa of mammals, frequently the stomata is found at the common junction of several endothelial plates, some irregular, granular, polyhedral cells which stain quite dark brown with Ag. NO<sub>3</sub>. There may be two, three, or more of these cells at one place, and they may be very small or very large. These cells surround the mouth of the vertical lymph channels which directly connect the peritoneal cavity with the subperitoneal lymph channels. They are some of the characteristic landmarks in the microscopic work of the peritoneum, and have been discussed and questioned from the days of Von Recklinghausen (1860) to the present time. They are the polemic of the peritoneum. They are known as stomata vera; observe that they may be open or shut. The place to begin to observe the stomata of the lymphatics is on the lymph sacs of frogs, where it is not easy to overlook them. In other portions of the peritoneum, then, the lymph sacs (*cisternae lymphaticae magnae*) one may certainly more regularly observe the stomata. To demonstrate them on the lymph sacs, stain the sacs *in situ* with  $\frac{1}{4}$  per cent. Ag. NO<sub>3</sub> for three to five minutes, after which snip off portions of the surface of the lymph sac and mount in glycerine. As the sunlight affects the silver salts the stomata will soon come typically into view. After carefully studying quite a number of specimens the student will learn to observe the invaginating of blood vessels by lymphatics. The wall of the blood vessels and the wall of the lymph vessels will show themselves distinctly, and by some exercise the blood vessels will be observed lying in the midst of the lymph vessel, invaginated like a dark rod. The lymph vessel is excentric and the blood vessel is concentric. In some specimens, as those of the amphibia, invagination is difficult to overlook. In some specimens, rabbits, rats, the author has noted it typically, but to the turtle we must look for typical and persistent invaginations of blood vessels by lymph vessels, and they are easily demonstrated by the lymph vessel surrounding or invaginating the blood vessel by the shape of the endothelia. The peculiar bendings of the lymphatics on each side of the blood vessel will aid in determining whether we are dealing with one excentric lymph vessel or a pair of lymphatics parallel to the blood vessel. The lymphatics of the various portions of the peritoneum may be



observed after considerable microscopical exercise in fresh specimens, but the rewards of indistinctness are not worth the pains. In the lymph vessels swim more or less lymph corpuscles.

It must be remembered that the lymphatics of the peritoneum are best seen if silvered while distended, as in recent death after active life. It is quite difficult to demonstrate the lymphatics of the mesentery unless brushed and silvered in situ. This is because the adjacent tissue does not hold the lymphatics potent. But one scarcely fails to demonstrate the lymphatics of the pleural surface of the diaphragm because the adjacent tissue does not allow the lymph vessels to collapse. Such facts demonstrate how delicate the lymphatics of the peritoneum are. In demonstrating the various regions of the lymphatics in the peritoneum we will frequently meet, especially in the gastro-splenic omentum, numerous patches of what Klein called germinal endothelium. Such patches stain highly brown with silver, and may partially develop into blood vessels and new endothelia.

The lymphatics of the peritoneum do not form a closed system, but originate from the interstitial tissue spaces and from the peritoneum by means of stomata. As Klein notes, the lymphatics develop from branched cells; they project from the endothelium of the lymph sac, and the lymphatic vessel is transformed into a cavernous structure. Lymph corpuscles originate from the endothelia and the branched cells which cross the lumen of the lymph sac. In the development and examinations of the peritoneal lymphatics, E. Klein makes a division into peri-lymphangial nodules and into endo-lymphangial nodules, but it seems to the author that these endo- and peri-lymphangial cords or nodules blend into and resemble each other.

The beginner may well be prepared for ill success in demonstrating the lymphatics of the fenestrated portions of the omentum of such animals as the cat, rabbit and rat, for it is so difficult to institute the proper amount of brushing. Often we brush too vigorously, when the subserous groundwork becomes disturbed. Seldom do we brush too gently. I had better success with the guinea-pig in demonstrating peritoneal lymphatics than any other mammal. But guinea-pigs are rather dear and scarce. The six-weeks'-old puppy furnished elegant specimens of lymphatics.

The cellular elements or the subperitoneal tissues of the peritoneum may be selected from any portion, but the best locality is from the region of the psoas muscle, or the extensive subserous tissue of the pelvis. Small bits may be mounted fresh in glycerine. If it be teased with needles the fibers become entangled and irregular, but if not traumatized the elements may be seen in their natural arrangements. Logwood and eosin are excellent stains, and acetic acid makes the tissue swell up,

affording opportunities to observe the tissue in different conditions. We may note in the subserous tissue the white fibrous tissue consisting of long, wavy, silken bundles. The bundles of fibrous tissue are delicate and jelly-like in appearance, in various thickness, coursing chiefly parallel to each other, or they are arranged in distinct, shining, plain or fasciæ. If the acetic acid is applied (5 per cent. to 10 per cent.) the fibrous bundles swell, become more homogeneous and may become almost invisible. The fibrous bundles cross and recross each other, split and reunite, forming various sized mesh-work. The fibrous bundles are held together by interstitial albuminous substance. The tissue may be macerated, i. e. : the interstitial substance is dissolved out so that the bundles split into their constituent fibres. For the purpose of maceration Na. Cl., 10 per cent. ; Ca. 2 H. O., 10 per cent. ; Ba. 2 H. O., 10 per cent. Potassium permanganate or bichromate of potassium may be used.

The elastic fibres, which are very numerous in the subserous tissue of many mammals, are characterized by the fact that they do not swell in acids and are not generally united into bundles. They occur as sharply defined threads coursing in an isolated state, sometimes straight, sometimes spiral and wavy. The elastic fibres form a mesh- or network, by repeated bifurcations and fusion. This may be well seen in the mesentery, mesocolon or parietal peritoneum of the rabbit. The rat shows the network exceptionally well, in which the elastic fibres are so numerous and dense that they almost form a perforated plane. In the intima of the arteries the elastic fibres really form a fenestrated membrane (Henle's), which can be demonstrated by stripping off fine shreds of the intima of the larger arteries with sharp scissors and fine forceps. The cellular elements of the subperitoneal tissue may be migratory, branched or fixed amoeboid cells.

An amoeboid cell may resemble a white blood corpuscle, consisting of finely granular protoplasm, contain one or more nuclei, exhibit motion, and are affected by reagents similarly to a white blood corpuscle. Again, an amoeboid cell may be large and coarsely granular. Both the large and small amoeboid cells may be typically found in the subperitoneal tissue. The branched cells of the subperitoneal tissue are flattened bodies consisting of finely granular protoplasm. Each possesses a nucleus, and a greater or less number of processes which are in continuity with each other, so as to form a network.

The various tissues of the peritoneum may be colored during life by injections into the veins or peritoneum. Various coloring matter may be selected and introduced, little by little, daily.

To study the effect of irritation on the peritoneum in a physiologic sense, the peritoneum should be injected with water containing fine grains of Berlin blue. Kill the animal  $\frac{1}{2}$  to several hours later, silver

the diaphragmatic serosa on the peritoneal side and mount very carefully in glycerine, when one will be able to observe the leucocyte in all conditions so far as regards its relation to the endothelia. The leucocytes may be observed (a) under the endothelia; (b) in the endothelial cleft; (c) in the lymphatics of the diaphragm containing the colored granules; (d) in the diaphragmatic serosa attempting to surround, digest or bury them.

To demonstrate the lymphatics of the diaphragm of the rabbit or guinea-pig is excellent. One can hardly fail to demonstrate the lymphatics on the pleural side, but it is more difficult to demonstrate them on the abdominal side.

To demonstrate the stomata the mesogaster of frogs is apt, or the gastro-splenic omentum and diaphragmatic serosa of rabbits is excellent.

To demonstrate the peritoneal nerves the lymph sac of the frog (amphibia) is apt, as well as the mesentery of cats and the folds of peritoneum stretching from the diaphragm to the stomach in rabbits.

To demonstrate the germinating endothelia the mesentery of frogs and the omenta of rabbits are good.

To demonstrate the cellular elements of the ground substance, the rabbit's omentum is good.

To demonstrate the membrana limitans the diaphragm of the rabbit is one of the best. It should lie in Muller's fluid several days, and then a very mild brushing and a gentle stream of water over the part is sufficient to prepare the specimens to mount in glycerine.

To demonstrate the elastic fibres, the mesentery of the rat, cat and rabbit are excellent.



## CHAPTER X.

### A RESUME OF THE PHYSIOLOGY OF THE PERITONEUM.

Certainly it is excellent discipline for an author to feel that he must say all he has to say in the fewest possible words, or his reader is sure to skip them; and in the plainest possible words, or his reader will certainly misunderstand them. Generally, also, a downright fact may be told in a plain way, and we want downright facts at present more than anything else.—*Ruskin*.

1. The peritoneum absorbs and secretes fluid. Solids will pass through it, especially the diaphragmatic portion, held in liquid suspension. In experiments the absorption is more manifest than the secretion unless special fluid known as hypertonic solution is employed.

2. In experiments the chief fact to observe is that fluids disappear from the peritoneal cavity.

3. The mechanism of peritoneal absorption must be studied structurally and functionally or histologically and physiologically.

4. As to structures in the peritoneum we have (a) the flat, smooth, nucleated endothelial cell, which has a hardened portion of protoplasm, the cover-plate; (b) the interendothelial space in which are located, (c) the stomata vera and (d) the stomata spuria.

5. The interendothelial space appears under the action of  $\frac{1}{4}$  per cent. Ag.  $\text{NO}_3$  to consist of two dark parallel lines crossed at short, irregular intervals by transverse protoplasmic anastomotic processes. The two parallel lines are on the borders or edges of the two adjacent cover-plates while the transverse processes are the protoplasmic projections which bind into colonies the endothelial cells. I have compared the interendothelial space to a railway in which the two parallel lines represent the rails and the transverse process the ties.

6. The stomata vera are apertures situated at the common junction of several endothelial plates. They are lined by polyhedral, protoplasmic granular nucleated cells which stain highly with Ag.  $\text{NO}_3$ . The typical location of stomata vera are on the serosa of the centrum tendineum. We attribute to the stomata vera dilatation and contraction, a control of fluids by means of sphincter. Some consider the stomata vera as centers of regeneration of endothelia to take the place of dying comrades.

7. The stomata spuria are structures located in the linear endothelial space. Some consider such structures as lymphoid corpuscles

(Virchow), others connective tissue cells projecting upward between two endothelial plates (Oedmansson), others as white corpuscles emerging between the cells, and still others as artificial products. By actual observation I know some are leucocytes passing from the subserous to the serous cavity.

8. The physiology of the peritoneum must be looked for in the interendothelial space by its dilatation and contraction. The cover-plates are perhaps not engaged much in the physiology. The hard, indurated metamorphized protoplasm of the cover-plate aids chiefly in a mechanical way to facilitate motion when aided by the visceral fluid secreted through the interendothelial space. However, the cover-plate doubtless plays a role in osmosis.

9. The paths of absorption of peritoneal fluid are either by way of the lymphatics (i.e., interstitial spaces) or blood vessels. The author claims that the fluid is first absorbed by the interstitial spaces.

10. The chief demonstrable locality of the absorption of finely divided granules of matter suspended in fluid is in the region of the diaphragm. The vast interstitial spaces or lymphatic channels in the diaphragm become rapidly filled with the fine particles of matter a few minutes after the material is injected into the peritoneum.

11. The colored granules are carried into the spaces free, but also by one or more leucocytes.

12. There is a stream of fluid in the peritoneum directed toward the diaphragm.

13. The peritoneum in the dead animal will absorb fluids similarly to the living for many hours after death. I have proved this definitely up to thirty-six hours. The animals absorbed during life as high as 10 per cent. of the body weight; 30 minutes after death they absorbed 6 per cent. of the body weight in 30 minutes.

14. The forces which are said to induce peritoneal absorption of fluids may be enumerated as follows: (a) Vital cell forces; (b) stomata; (c) imbibition; (d) filtration; (e) intra-abdominal (mechanical) pressure; (f) osmosis.

The most significant factors are, perhaps, osmosis and filtration. In experiments on the peritoneum to test its absorptive or secretory powers solutions of certain standards are used, and known as hypotonic, isotonic or hypertonic. Isotonic solutions have the same osmotic pressure as the blood. They range, according to different authors, from 0.72 per cent. to 0.92 per cent. Na.Cl. solution. Hypotonic solutions will be less than 0.72 per cent. Na.Cl. solutions. Hypertonic solutions will be above 0.92 per cent. Na.Cl. solution. According to the law of osmosis, hypotonic solutions, i.e., below 0.75 per cent. Na.Cl. solution, should absorb rapidly, which they do. The isotonic solution, i.e., 0.72 per

cent. to 0.92 per cent. of Na.Cl., should not absorb at all, or but slowly. Hypertonic fluids do not absorb by attracting fluids from the blood. In my experiments this was generally the case.

15. Because chemic or thermic injuries do not stop peritoneal fluids from absorption, because the dead peritoneum absorbs fluids many hours after death, we must likely exclude "vital forces," the cells as a process of absorption. However, Thoma claims that the surface of the vascular endothelia possesses a secretory power.

16. Imbibition, molecular or capillary, being of a limited process does not account for the large volume of fluid the peritoneum will absorb.

17. Filtration comes under mechanical or intra-abdominal pressure.

18. As the stomata are disputed structures, space is too short to discuss them here. The structures known as stomata are not disputed so much as their interpretation. In regard to later writers, Kolossow and Muscatello deny the existence of stomata; Beck, Notkins, Klein and others assert with equal vigor their existence. The doubting investigators seem to simply make different interpretations of such structures.

19. Stomata vera are vertical canals, lined by granular polyhedral protoplasmic cells which directly connect the peritoneal cavity with the subperitoneal lymphatics.

20. The peritoneum is a lymph sac. Its origin is due to fluid pressure and independent motion of viscera and body wall.

21. Its endothelial plates rest on a vast lymph bed consisting of valveless interstitial spaces and valved lymphatic channels.

22. With but slight exception, the lymphatics of the peritoneum (and those of the intestines) empty into the thoracic duct (lower).

23. In mammals the chief and characteristic lymphatics of the peritoneum lie in the diaphragm or centrum tendineum. The lymphatics of the diaphragm, vast in extent, terminate in two posterior trunks which empty into the thoracic duct, just above the diaphragm, and into two anterior trunks which accompany the internal mammary arteries.

24. It is on the diaphragm (1861) where Von Recklinghausen first observed the disappearance of milk globules (and other matter) in whirls and eddies through the stomata of the serosa of the centrum tendineum.

25. The turtle (amphibia) is one of the best of animals to show vast interstitial subperitoneal spaces, and also large peri-vascular spaces. The turtle's peritoneum shows typically that blood vessels lie either in interstitial spaces or peri-vascular spaces—never in contact with the serosa of the peritoneum. This structural fact plays a significant role in



rapid primary absorption, and indicates that the intestinal spaces are apt to absorb the first excess of fluid in the peritoneum.

26. The lymphatic system of the subperitoneal tissue consists of (a) intestinal spaces, (b) lymphatic capillaries and (c) valved trunks.

27. The fluid in the interstitial spaces consists of (a) the fluid transuded from the blood capillaries, (b) the fluid minus the material required to nourish the cells, and (c) the effete material of living tissue.

28. Ligation of the thoracic duct retards distinctly the absorption of peritoneal fluids. (In my experiments about 20 minutes.)

29. Ligation of thoracic duct retards the transpiration of salts from the peritoneal cavity through the circulation into the bladder about 20 minutes.

30. With free or non-ligated lymphatics salts become transported from the peritoneal cavity through the circulation into the bladder in 7 minutes.

31. With the recognized rapidity of peritoneal absorption in extensive and dangerous hemorrhage peritoneal injection might save life.

32. In 30 minutes during life the peritoneal cavity will absorb as high as 10 per cent. of the body weight. After death, say a few hours, during 30 minutes, the peritoneum will absorb 6 per cent. of the body weight.

33. The rapid fluid absorption by the peritoneum teaches against irrigation in laparotomy, from the fact that the germs would become widely and rapidly distributed.

34. White blood corpuscles appear to be continually passing into and out of the peritoneal cavity.

35. The function of the leucocyte may be tested by the injection of fluid containing solid particles in suspension into the peritoneal cavity. A few minutes after such fluid is injected into the peritoneum the leucocytes begin to swarm on the surface of the peritoneal endothelia. By observation in different stages of the experiments one may note the leucocytes distinctly beneath the cover-plate in various positions in the interendothelial space. Occasionally we may note either end or the middle portions of the leucocytes clamped between the adjacent borders of the cover-plates, or finally the leucocyte may be seen on the peritoneal surface.

36. The function of the leucocytes seems to be (a) to surround the foreign body and transport it into the subserous lymphatics; (b) to bury the foreign body; (c) to digest it, or (d) to sterilize it by imprisoning and isolating it by exudates. If one leucocyte be unable to bury a foreign body, many will come to the aid.

37. Whatever tends to induce migration of one leucocyte seems to call out swarms of leucocytes.

38. The leucocytes act like a body-guard to the peritoneum. At a moment's notice (irritation) the swarms of leucocytes emerge to protect invasions against the peritoneum, viz., by digestion, imprisonment, transportation or sterilization of the foreign, be it vegetable germ or inorganic particle.

39. The *membrana limitans* is a thin ground-glass-like membrane, resembling the wall of a soap bubble, on which the peritoneal endothelia rest. Brushing the endothelia off leaves a pit-like depression in it.

40. The *membrana limitans*, or basement membrane, is found to be perforated by numerous apertures on the diaphragm (*centrum tendineum*) only, and this anatomical fact serves as physical or mechanical explanation, why the chief, and perhaps only, locality of the peritoneum which absorbs solid particles held in liquid suspension is the diaphragm.

41. The enormous activity of the diaphragm in absorption makes it an intensely dangerous locality for infections.

42. The dangerous areas in peritonitis are those places of active absorption, as the diaphragm and small intestines, for absorption and infection kills, while peritonitis saves life. The benign areas, the safe areas of peritonitis, are those areas of slow absorption where exudates form. The benign areas of the peritoneum are those of the large intestine, i. e., the pelvic, the appendicular and gall-bladder region.

43. The vast, loose shifting and spongy bed of snow-white connective tissue on which the peritoneum rests, especially in the dorsal region, endows the peritoneum with an elastic accommodation for the movement and shifting of organs. It serves as a buffer to lessen trauma. It is endowed with much capacity to circumscribe and tolerate local infectious material.

44. The capacity of the subperitoneal tissue to split and resplit into fine, smooth planes allows the infectious fluids to move in many directions for accommodations and escape. The fluids penetrate in the direction of least resistance, which is well recognized by clinicians as to the movement of pus in the pelvis and psoas muscle.

45. The histology of the subperitoneal tissue teaches why the viscera can shift as in visceral ptosis. The displacement of the viscera seems to be chiefly checked by the two factors, (a) the blood vessels (arteries) and nerves and (b) the *membrana mesenteric propria*.

46. The automatic power of the peritoneum to secrete the proper amount of fluid to facilitate motion of viscera is a very delicate physiologic process.

47. A difference in function between an endothelial and an epithelial cell is that an epithelial does not normally secrete albumen while an endothelial cell does.

48. Epithelial and endothelial cells are both alike bound in colonies of protoplasmic processes.

49. The omentum acts as a drain to the peritoneal cavity.

50. The omentum is the great protector against peritoneal infectious invasions. It builds barriers of exudates to check infection. It is like a man-of-war, ready at a moment's notice to move to invaded parts. It circumscribes abscesses, it repairs visceral wounds and prevents adhesions of mobile viscera to the anterior abdominal. It is like a moving sentinel, whose beat is the whole peritoneal cavity. It is the surgeon's friend, covering up the evil his hands have wrought. It is a diagnostic aid, directing the surgeon to the original seat of peritoneal diseases where it first contracted adhesions. It closes intestinal wounds. The omentum is an area of peritonitis, not an area of infective absorption. It resists infectious invasions by typical peritoneal exudates, and not by succumbing to absorbed sepsis. Comparative anatomy teaches us that the omentum is not for the purpose of keeping the intestines warm. It is one of the first localities for excessive accumulations of fat, and one of the first places to disappear in emaciation. It is a storehouse for fat. It is a director of peritoneal fluids, a peritoneal drain.

51. If the peritoneum be silvered very lightly for a short time, say,  $\frac{1}{4}$  per cent. for a minute, it can be resilvered and also treated with other reagents, fixing or coloring it.

52. The peritoneum may be viewed as a joint lined with synovial membranes. It is a joint of vast physiologic activity.

53. Endothelium varies in shape and kind, according to the organ on which it is located. In general it is more regular in outline if it be on an organ which is quiet, i. e., if it rest on an unmovable base it is quite regular in shape and outline. If the endothelia rest on a movable base, as many of the viscera, the endothelia take a shape which elongates in the direction of expansion and contraction. In the vessels, however, the endothelia are elongated in the direction of the fluid stream. On the expanding and contracting bowel the endothelia generally elongate in a direction transverse to the faecal current, i. e., transverse to the bowel. The very great irregularity of the shape of the endothelia in the lymph spaces is doubtless due to the irregularity of fluid expansion and the indefinite direction of its current at various periods. The endothelium over the ovary assumes a germinal character. It assumes a columnar shape. Germinating endothelia in general are not so flat or squamous as the common peritoneal endothelia; are smaller and rounder in outline than adjacent endothelia. The endothelia over the kidneys are comparatively uniform in size and hexagonal in contour.

54. The peritoneum is equal in area to that of the skin. Its absorptive power is far greater than the skin.



55. The peritoneum has a limited power to resist septic germs. At present we have no standard by which the power may be measured. The power to resist septic germs is slightly different in different animals. The peritoneum of the pig, rabbit and cow resist considerable quantities of septic organisms. The mare's peritoneum is so sensitive to septic germs that laparotomy on her is almost always fatal. The dog and man are about equal in their power to resist peritoneal invasions of pathogenic germs. Some, however, claim that the peritoneum of the dog resists more than that of man.

56. The peritoneum presents the most favorable conditions for healing.

57. The different degrees of vulnerability in the peritoneum, in all probability, rest on its close relation to its lymphatic structure.

58. The peritoneum is peculiarly sensitive. The nerves of the peritoneum are vast in extent and number. They are (a) medullated; (b) non-medullated and (c) various forms of terminations as the Vater-Pacinian corpuscle. The kitten is one of the best animals to demonstrate the peritoneal nerves. Acetic acid and gold chloride are the best reagents.

59. The numerous terminations and vast area of nerves in the peritoneum account for the profound shock which is intimately and closely associated with the calibre of vessels, controlled chiefly by the sympathetic nervous system. Reflexes from the peritoneum are most profound on distant viscera.

60. Peritoneal surface lessens with ascending scale of mammalian life. Man has, proportionately, the least amount of peritoneum of mammals. Man's omentum majus is much smaller than that of many mammals.

61. Through ages of evolutionary processes of infective invasion, the pelvic, appendicular and gall-bladder region (the region of the large intestine), has acquired a physiology which resists the infectious germs, in the common regions of peritonitis.

## EXPLANATION OF THE FIGURES.

*Taken from an article by Dr. G. Muscatello, of Turin, Italy. (Virchow's Arch. Bd. 142, 1895.)*

- FIGS. 1 and 2.—Isolated endothelial cell from normal peritoncum of man.  $\times 1,250$ .
- FIG. 3.—Endothelial cells from the serous covering of a guinea-pig's spleen. Profile view  $\times 600$ . (Prepared with osmic acid and tannin, glycerine.) Note cilia.
- FIG. 4.—Endothelial cells from peritoneum of a guinea-pig's diaphragm on the peripheral part. Observe the long processes on the cells.  $\times 560$ .
- FIG. 5.—Endothelia from the centrum tendineum of rabbit. Observe that Dr. Muscatello has drawn no openings, stomata, between the cells, which is in accord with his opinion.  $\times 560$ .
- FIG. 6.—Endothelia from the peritoneal diaphragm of a healthy dog. One can observe a whitish oval space at the common junction of three cells, which is covered by a superficial transparent layer, i. e., the cover-plate of the endothelial cells.  $\times 1,250$ .
- FIG. 7.—Endothelia from the peritoneal serosa of the diaphragm of a healthy dog. One can observe leucocytes between the endothelial cells.  $\times 275$ .
- FIG. 8 A.—Peritoneum from diaphragm of man, natural size a, peritoneal covering of muscle layer whose zona peritendinea is distinguished with Zp. (b), peritoneum of the centrum tendineum, numerous perforations in the zona peritendinea, with the exceptions of the peritoneal covering of the large vessel V.
- FIG. 8 B.—Part of the zona peritendinea, three times enlarged. Wide apertures between the bundles of the stratum vorticulare running in different directions. This figure is from Salvioli and Bizzozero. Enlarged 350.
- FIG. 9.—The membrana limitans from the peritendineum of the diaphragmatic peritoneum. It has groups of perforations in three localities. This figure is after Bizzozero and Salvioli. Enlarged 350 times.
- FIG. 10.—A piece of peritoneum from the zona peritendinea; a, a, small lymphatic spaces; b, b, bundles of net formed layers which surround the spaces like rings; c, c, c, connective tissue bundles of supporting tissue d, which in part run over the lymphatic spaces; e, a piece of the membrana limitans with many perforations. E, is not well marked, but it is in the upper left-hand corner of the figure. The rest of the membrana limitans was accidentally removed during the preparation of the specimen. This figure is from the excellent labors of Bizzozero and Salvioli. It is enlarged 50 times.
- FIG. 11.—Membrana limitans from the peritoneal diaphragmatic serosa of man. Delicate furrows border the flat niches in which the endothelial cells were embedded. Enlarged 600 times.
- FIG. 12.—Leucocytes beladen with smaller and larger sized granules. Enlarged 560 times.
- FIG. 13.—Large sized granules beladen with several leucocytes. In b, the leucocytes contain still smaller granules.  $\times 560$  times.
- FIG. 14.—Endothelium from the diaphragmatic peritoneum of dog, during the absorption of large granules. Large white openings may be seen between the endothelial cells. Enlarged 1,250 times. The large, oval reticulated nucleus contain black nucleoli.
- FIG. 15.—Another place of the same preparation as Fig. 14. Leucocytes and granules in the act of wandering through the endothelial layer. Enlarged 350 times.

NOTE.—Some copies of my figures in this book, as well as abstracts, have appeared in a few journals. The Medical Record published some. —[Ed.]







# INDEX.

Asellio.....	2, 3	Beck.....	246
Arnold.....	13, 131, 184, 855, 359	Birdsall.....	248
Albumen.....	44, 357	Bajard.....	255
Ascites.....	80	Bourgery.....	255
Affannasiew.....	85, 89, 125, 226, 246, 359	Berkely, Henry J.....	256
Absorption.....	86, 104, 145, 147, 281, 337, 339	Blood stream.....	339
.....	346, 371, 394	Blood plasma.....	339
Apertures.....	99, 132, 133, 203, 218, 240	Blood serum.....	343, 380
Anastomosis.....	101, 262	Blood serum of ox.....	344
Appendicitis.....	118	Blood serum of sheep.....	344
Abdominal Serosa.....	124	Blood serum of hare.....	344
Aeby.....	154, 194	Billroth-Meissner's plexus.....	
Auerbach.....	154, 194, 226, 246, 359	Berlin Blue (injections of).....	348
Appendicæ epiploicæ.....	161	Blood capillaries.....	352
Amphibia.....	177	Bladder.....	356
Adventitia.....	177	Capillaries of diaphragm.....	150
Aorta, Thoracic and Abdominal.....	177	Cortical.....	158
Alferow.....	194	Colotomy.....	171
Arteries, Internal Mammary.....	210	Conheim.....	182, 184
Amphibian lymph sacs.....	217	Capillaries, non-valved.....	204
Auerbach's plexus.....	256, 261	Centrum tendineum.....	210, 256, 374
Axis cylinder.....	259, 260, 264	Corpuscle, lymph.....	223
Anat. Minute.....	278	" connective tissue.....	223
Absorption (gas, oil, fluid).....	291	Capillary lymphatic fields.....	228
Absorption (by vital course) stomata, intra-		Cisternæ lymphaticæ magnæ.....	236, 258
abdominal pressure, filtration, imbi-		Capillaries of intestines and spleen.....	338
bition, osmosis.....	338	Cyon.....	257
Absorption (of peritoneal fluids).....	339, 346, 349	Coagulation.....	264
.....	356, 375	Cyon.....	273
Absorption (of serous fluid).....	345	Conclusions (of absorption paths).....	288, 289
Adler and Meltzer.....	354, 358	Capillary tension.....	338
Amputated limbs.....	355	Capillary pressure.....	344
Absorption of proteids of tissue.....	357	Cohnstein.....	351, 357, 358
Absorption by lymphatic system.....	357	Cells (vital action of).....	359
Absorption of salt.....	357	Capillaries (lymph or blood).....	359
Bartholin.....	3	Chrzenszczewsky.....	358
Bichat.....	8, 26, 30, 42, 280	Conclusions on technique of peritoneum.....	392
Brechet.....	8	Conclusions on Peritoneal absorption.....	393 to 399
Burdon-Sanderson.....	12, 82, 125, 246, 359	Chyle.....	9, 44
Bruns.....	13	Clark.....	4
Brecke.....	27	Cruikshank.....	6
Bowman.....	30, 131	Cruveilhier.....	9
Bizzozero.....	30, 125, 218, 246, 359	Cisterna Lymphatica Magna.....	9
Blood vessels.....	39, 278, 362	Cells.....	28
Bordeau.....	42	" connective tissue.....	29, 31, 43, 103
Bizzozero.....	86, 148, 296	" fibrillar.....	106
Bands, pleuritic.....	105	" endothelial.....	29, 48, 76, 378
Bands, peritonitic.....	105	" wandering.....	31, 116
Bichat.....	108	" branched.....	31, 106
Beale & Schultze.....	114	" vacuolated.....	31, 96, 106, 116
Brinton.....	131	" pigment.....	31, 162
Blood vessels.....	177, 224, 233	" fat.....	31, 106, 159
Binz.....	184	" muscle.....	31
Bottcher.....	187	" nerve.....	31, 106
Blood vessels, development of.....	200	" vascular.....	31
Blood vascular capillaries.....	239	" elastic tissue.....	31, 32
Brucke.....	240	" lymphoid.....	44, 97

- Cells, granular polyhedral nucleated.....77, 85  
 " granular.....114, 115, 248  
 Cilia.....20, 38, 39, 77, 98, 220, 222  
 Chyme ..... 46  
 Cement-substance..... 72  
 Cover-plate.....40, 41, 73, 75, 101, 102, 128  
 Cleft..... 78  
 Canal, vertical.....80, 127  
 " interendothelial..... 100  
 Centrum tendineum of diaphragmatica  
 peritoneum 93, 120, 122, 123, 128, 138, 172  
 Cords..... 96  
 Connective tissue corpuscles.....98, 116  
 Cuvier ..... 122  
 Cetacea..... 122  
 Chrzozozinsky.....125, 194, 246  
 Cell-fat, Use of in Peritoneum..... 160  
 " spaces..... 207  
 " borders..... 240  
 Ductus agnosi..... 4  
 Desault..... 8  
 Diaphragm..... 21, 33, 34, 76, 120, 222, 305  
 Dujardin..... 28  
 Dubar.....80, 86, 246  
 Dogiel.....81, 82, 125, 226, 246, 359  
 Dybkowsky.....82, 85, 125, 226, 246, 359  
 Deckhuyzen..... 101  
 Diaphragmatic serosa.....104, 283  
 Diaphragmatic lymphatics.....138, 139, 140  
 Diaphragm nerve supply..... 120  
 Diaphragm, function of.....122, 287  
 Diaphragm, experiment on ..... 148  
 Donders..... 187  
 Drainage..... 222  
 Diaphragm (stream toward)..... 287  
 Diaphragm (locality of absorption)..... 308  
 Dropsical conditions of the peritoneum .... 350  
 Dubar and Remy..... 359  
 Diaphragm (absorption of)..... 369  
 Demonstration of lymphatics of diaphragm. 385  
 Diaphragm (activity of)..... 397  
 Erasistratus..... 1  
 Ebers..... 1  
 Eustachius..... 2  
 Endothelia.....18, 19, 29, 37, 75, 82, 85, 92, 93  
 94, 102, 166, 178, 219, 238, 242, 398  
 Epidermis ..... 26  
 Epithelia.....37, 44  
 Epiblast..... 43  
 Endothelial plate.....91, 180, 202  
 tube ..... 203  
 contraction and expansion.....  
 arrangement of.....92, 94  
 Endothelia, growth of..... 95  
 Eberth.....154, 194  
 Endothelia, peritoneal..... 190  
 blood vascular..... 190  
 lymph vascular..... 190  
 Germinating..... 398  
 spindle-shaped..... 217  
 Endothelia (shape, lineaments, grouping)... 240  
 diameter..... 242  
 Elastic fibre..... 258  
 Endothelia (nucleus)..... 278  
 Experiments (of author).....318, 363  
 Experiments on dog..... 348  
 rabbits..... 348, 369  
 Experiments on guinea-pig..... 348  
 cat..... 348  
 Endothelial cells.....348, 355  
 Endothelial membrane..... 353  
 Experiments of Adler and Meltzer.359, 360, 361  
 .....367, 368  
 Experiments on animals.....364, 365, 366  
 Experiments of Starling and Tubby.....370, 371  
 Fohman.....9, 10  
 Frey.....11, 226, 246  
 Frommann..... 28  
 Fallopian tubes.....37, 44  
 Foa.....86, 99  
 Fat globules.....92, 152  
 Fibres..... 109  
 elastic .....110, 112, 113  
 Fatty tissue..... 118  
 Fuhrer ..... 154  
 Follicles..... 158  
 Frey..... 158  
 Flemming. .... 160  
 Filtration....209, 338, 339, 355, 358, 362, 394  
 Fohman..... 212  
 Fluid.....212, 214, 224, 231, 349  
 Finkham.....273, 274  
 Friction of Serous Membranes..... 280  
 Fluid (colored) ..... 296  
 Function (of endothelia), peritoneal, vascular  
 and lymph..... 304  
 (of interstitial spaces).....309, 310  
 Filtrate hypothesis..... 340  
 Fluid (ascitic)..... 340  
 Fick, A..... 340  
 Fluids (intra-vascular and extra-vascular) ... 344  
 Fluid (dropsical)..... 350  
 Fibres (elastic, muscular)..... 354  
 Function of interstitial spaces ..... 354  
 Frye..... 359  
 Galenus (Galen)..... 1  
 Ganglia..... 2  
 Glisson.....2, 7  
 Goodsir.....11, 131  
 Gerber..... 13  
 Grew ..... 27  
 Glisson's capsule..... 172  
 Goluben..... 182  
 Gunning ..... 187  
 Glands, mammary.....215, 354  
 salivary.....215, 354  
 testicular..... 215  
 Gegenbaur ..... 225  
 Gold chloride..... 258  
 Gaskell..... 274  
 Granules (colored) ..... 293, 351, 357, 391  
 Glands (pyloric)..... 295  
 (lumbar)..... 295  
 Glands (mediastinal)..... 351  
 Glands (sweat)..... 354  
 Heliopolis..... 1  
 Harvey.....2, 3  
 Hoffman.....3, 7, 194, 274  
 Hewson.....5, 6, 7, 339  
 Hunter, John.....5, 7  
 Hunter, Wm.....5, 7  
 Haller.....7, 255  
 Henle .....9, 10, 20, 109



Herbst.....	10	Lymph channels.....	77, 81, 128, 222, 228, 229
Hyrthl.....	11	capillaries.....	214, 224, 234, 235, 240
His.....	12, 37, 39, 43, 157, 192, 225, 240, 359	Lawdowsky.....	82, 80, 101, 125, 184, 240, 305, 359
Haeckel.....	27	Lymph sacs.....	86, 217
Hooke.....	27	spaces.....	144, 173, 216
Heitzmann.....	28	sinus.....	96, 98, 142
Hiswas.....	43	fluid.....	234
Hypoblast.....	43	Ligamentum latum.....	111
Hermann.....	82, 85, 99, 125, 246	peritonei.....	114
Hertwig, Oscar.....	120	Lymph, corpuscles.....	116, 224
Hyrthl.....	158	trunks.....	144, 231
Hernia.....	160	Layers of centrum tendineum.....	123
Hering.....	182	Leipzig Physiologic Institute.....	125
Hamburger.....	337, 339, 345, 346, 351, 356, 359, 369	Lymphoid cells.....	128
Hale.....	339	Lymphatics of diaphragm.....	148, 150, 173
Heidenhain.....	342, 343, 346, 350, 351, 356, 359	system.....	206
Interendothelial space.....	31, 33, 34, 58, 65, 198, 238	Lymph nodes.....	156
line.....	34, 65	gland.....	156
tendinous spaces.....	123, 126, 142	cleft.....	228
Injection.....	152	stream.....	233, 292, 373
Interstitial space.....	204, 210, 214	Lessing.....	154
Imbibition.....	209	Lymphangial tracts.....	169
Interstitial space—subperitoneal.....	211, 234	Langerhans.....	194
Intestines, small.....	222	Loret.....	194
Infection.....	251	Lymphatics (origin of).....	206
Inzani.....	255	Lacuna.....	206
Inflammation.....	256	Leydig.....	219
Intestinal mucosa.....	338	Ligamenta lata.....	222, 258
Imbibition (capillary, molecular).....	340, 346	Line of lymph endothelium.....	240
Imbibition.....	355, 395	Lamination.....	266
Joyliffe.....	3, 4	Lymph nodes.....	278
Juice canals.....	223, 249	Lazarus-Barlow, W. S.....	338, 344, 357
Jullien (L.).....	255, 272	Leathes, J. B.....	343, 344, 345, 351, 357
Jugular vein.....	359	Ligation of innominate veins.....	347, 358
Kolossow.....	359, 5, 24, 29, 37, 68, 85, 87, 100, 125, 186, 226, 246	Ligation of lymphatics.....	348
Krause.....	10	Lymph channels.....	348, 355
Koelliker.....	11, 132, 158	Ligation of thoracic ducts.....	351, 358, 360
Klein.....	28, 30, 68, 81, 82, 85, 96, 126, 226, 246, 359	Lymph capillaries.....	352
Kidneys.....	44, 118, 356	Lymph sinuses.....	353
Karyokinesis.....	98	Lymphatic Pathways.....	357
Key.....	187	Leucocytes (function of).....	396
Kitt-substance.....	197	Leucocytes (migration of).....	396
Kundrat.....	359	Mesentery.....	2, 33, 95, 258
Köhne.....	370	Malpighi.....	3, 255
Lacteals.....	1, 2, 44, 46, 156	Meckel.....	5, 7, 9
Liver.....	2, 122, 222	Muscattello.....	5, 28, 30, 69, 79, 80, 82, 85, 87, 125, 226, 246, 292, 359
Lymph.....	3, 38, 232	Monroe.....	5, 6, 7
Lieberkuhn.....	4	Mascagni.....	89, 212, 217, 226
Lauth.....	8	Muller.....	26
Lobstein.....	8	Mirbel.....	27
Lymph hearts.....	9	Mohl.....	28
Luschka.....	11, 109	Membrana Limitans.....	30, 31, 102, 106, 120, 130, 132, 134, 174, 252, 296, 307, 397
Ludwig.....	13, 28, 82, 85, 93, 226, 246, 339, 340, 349, 359	Mesoblast.....	42, 48, 117
Lymph vessels.....	21, 23, 32, 39, 140, 156, 204, 360	Mesothelium.....	48
Leydig.....	27	Mesenchyma.....	48
Leuwenhoek.....	27	Maffucci.....	87, 125, 246, 286, 359
Leucocytes.....	38, 82, 102, 184, 203, 284, 250, 252, 253, 290, 302, 305	Membrana mesenteria propria.....	114, 170, 172, 176
Lamina superficialis.....	40, 41, 46, 51	Membrane, basement.....	125, 397
Lamina inferior.....	40, 46, 51	serous.....	160
Line, Interendothelial.....	75, 103, 178, 202, 244	germinal.....	131
		Mesogaster, frog.....	154
		Medullary tubes.....	158
		Metchnikoff.....	187, 251
		Meyer, Jos.....	218
		Miller, J.....	23

- Medullary sheath.....264, 265  
 Merkel.....272  
 Matler (colored).....286, 349  
 Mesenterium (not perforated).....307  
 Microscopical exam.....320, 321  
 Magendie.....370  
  
 Nuck.....7  
 Nucleus.....29, 40, 53, 59, 97, 116, 262, 265  
 Nodules.....95, 107  
 Nerve supply.....158  
 Nutrition.....222  
 Notkins.....226  
 Nerve network.....256  
 Nesterowsky.....256  
 Nerves, Peritoneal.....257, 263  
     Medullated.....264, 265, 267  
     Non-Medullated.....  
     Remak's nerve fibres..261, 262, 265, 267  
     Vater-Pacinian corpuscles.....  
     Nerve cells.....  
 Neurolemma.....264, 265, 268  
 Nodes of Ranvier.....264, 266  
 Nerve Endings.....274  
 Nerve Peritoneal network.....276  
 Nerve-Axial fibres.....276  
 Nerve Periphery.....276  
 Nerve Pain.....277  
 Nikolsky.....378  
  
 Omentum....33, 76, 95, 137, 161, 222, 250, 258  
     .....398  
 Osmosis..44, 192, 209, 341, 342, 345, 355, 394  
 Oedmansson.....85, 148, 192, 246, 359  
 Outlines of peritoneal endothelia.....90  
 Oedema.....91  
 Ova-sac.....221  
 Orlow.....346, 350, 351, 356, 357, 378  
 Osmosis of glucose.....357  
  
 Paraschites.....1  
 Ptolemy.....2  
 Peritoneum.....1, 2, 5, 6, 222  
 Pavia.....2  
 Pancreas.....2  
 Pequet.....3, 13  
 Pinel.....8  
 Panizza.....9, 217  
 Pacinni.....11  
     corpuscle.....11  
 Protoplasm.....27, 28, 40  
 Purkinje.....28  
 Paladino.....37  
 Pflueger.....44  
 Peritonitis.....56, 80, 138  
 Peritoneum.....  
     Endothella.....10, 14, 22, 104, 355  
     Function.....13, 250  
     Histology and Physiology of....14  
     Preservation of.....21  
     Membrana llultans.....23  
     cavity.....143  
     ground substance.....164  
     nerves of... ..255, 257, 258  
     " ".....271  
 Peritoneal (plates).....38, 39, 103  
     (absorption).....299, 394  
     membrane.....55  
     fluid.....80, 128, 283  
     serosa.....104, 222  
  
 Peritoneal cavity.....218, 252, 359  
     bed.....245  
 Peritoneum, humidity.....280  
     motion.....280  
     friction.....280  
     polish.....280  
     absorption.....286, 377  
     joint cavity.....290  
     (fluid composition).....290  
     (preparation).....298  
     (currents).....300  
     (contraction, distension).....308  
     lymp or interstitial space.....308  
     irritation.....315  
     (physiology of).....315, 316  
 Peritoneum (methods of absorption)....333, 334  
 Peritoneal absorption (by mechanical pres-  
     sure).....337  
 Peritoneum (Rabbit's).....337  
 Processes.....73, 202  
 Precipitate.....74  
 Puerperal Fever.....80  
 Pia Foa.....85, 125  
 Pig embryo.....92  
 Patches.....96  
 Peri-lymphangial.....97  
 Pinel.....108  
 Pus.....117  
 Pica-duodeno-jejunalis.....121  
 Pericardial cavity.....121  
 Pillars of Uskow.....121  
 Pores.....134  
 Puerperal peritonitis.....138  
 Puncture method.....152  
 Ptosis.....161  
 Proliferation.....163  
 Patches.....168, 169  
 Poisonille.....187  
 Ponfick.....194  
 Pouyes.....194  
 Plexiform.....207  
 Plasmatic vascular tubes.....212  
 Perforation.....218  
 Perivascular.....236  
 Plates, stomatal.....246  
 Pflueger.....250  
 Physiology.....278, 315, 316  
 Protoplasm (retraction).....301  
     (reproduction centers).....301  
 Pressure (capillary, peritoneal).....338  
 Plexus pampiniformis.....340  
 Paths of absorption of peritoneal fluid..347, 350  
     .....352, 353, 394  
 Potassium ferrocyanide.....347, 359  
 Paths of interstitial fluids.....354  
 Pressure (mechanical).....357  
 Pia.....359  
 Prochaska.....370  
  
 Rudbeck.....2, 3  
 Ruysch.....7  
 Recklinghausen..7, 12, 13, 79, 85, 80, 147, 148  
     .....226, 246, 284, 359  
 Rudolph.....9  
 Rindfleisch.....13  
 Reagents, silver nitrate.....18, 30  
     logwood.....20, 24, 29, 30  
     Auric chloride.....255  
     Osmic acid.....24, 100

- Reagents, Tannin..... 24  
 Acetic acid.....61, 62, 87, 255  
 Muller's fluid..... 134  
 Eosin, acid fuchsin..... 135  
 Berlin blue..... 138  
 Formaline..... 140  
 Chloride of Platinum..... 255  
 acid chromic..... 255  
 Remak.....27, 269, 270  
 Ranvier.....29, 30, 31, 32, 82, 85, 98, 125, 246  
 .....200, 359  
 Remy.....85, 86, 125, 246  
 Regeneration of endothelia..... 94  
 Rings..... 99  
 Retroperitoneal abscess..... 117  
 Radjewsky.....125, 148, 359  
 Reichert.....132, 255  
 Retro-sternal glands..... 144  
 Retzli..... 170  
 Reitz..... 194  
 Robin..... 246  
 Radiation..... 266  
 Settala..... 2  
 Salzmann..... 4  
 Stomata...6, 7, 14, 148, 154, 209, 218, 224, 248  
 .....240  
 Sheldon..... 8  
 Sappey..... 11  
 Stomata vera..17, 23, 31, 32, 60, 74, 76, 78, 81  
 .....126, 246  
 spuria..23, 31, 60, 74, 88, 126, 127, 128  
 .....182, 246, 248  
 Schleiden..... 26  
 Schwann.....26, 258, 270  
 Schultze.....26, 27  
 Stricker.....27, 182, 187  
 Sarcode..... 28  
 Stomata, interendothelial..... 30  
 Space, interendothelial.....73, 75, 76, 86  
 perivascular..... 225  
 Silver nitrate..... 74  
 Substance, interendothelial.....75, 76, 86  
 endo-lymphangial..... 226  
 Stomata vera, functions of..... 80  
 Schweigger-Seidel..81, 82, 85, 93, 125, 226, 240  
 .....246, 359  
 Shape of endothelia..... 89  
 Source of germinating cells..... 96  
 Subbotin..... 101  
 Strands, fibro-elastic..... 116  
 Septum transversum..... 122  
 Salvioli..... 125, 246  
 Serosa, pleural..... 130  
 peritoneal.....130, 174  
 Sympathetic nerves..... 170  
 Sheath, subperitoneal..... 172  
 Subbotin..... 180  
 Stigmata..... 182  
 Schklarewsky..... 187  
 Sweat..... 215  
 Sacculation..... 216  
 Spleen..... 222  
 Subserous lymphatic vessels..... 244  
 Septic..... 254  
 Sepsis..... 256  
 Schmidt-Lauterman..... 264  
 Sensation..... 277  
 Stomata.....277, 284, 353, 355, 395  
 Stomata (anatomical structure of)..... 301  
 (accidents from reagents)..... 301  
 Vera..... 393  
 Spuria.....302, 393  
 Generative centers..... 307  
 Diaphragmatic..... 370  
 Regulate Perit. currents..... 308  
 Subperitoneal Tissue.....310, 311, 359  
 Spaces (interstitial).....311, 317, 348  
 Starling.....338, 343, 346, 350, 355  
 Spaces (interendothelial)..... 339  
 Spaces (intravascular)..... 340  
 Solutions (isotonic and hypertonic).....342, 344  
 Spaces (intertendinous)..... 349  
 Starling and Tubby.....349, 359  
 Starling's (experiments)..... 352  
 Spaces (intercapillary)..... 353  
 Tadino..... 2  
 Thoracic duct.....3, 156, 224, 345, 347, 359  
 Tledemann..... 9  
 Teichmann.....12, 158, 246  
 Tissue, Subserous.....15, 20, 31, 116, 120  
 white fibrous.....16, 106, 109  
 elastic.....16, 106, 110  
 mesoblastic..... 16  
 areolar.....106, 118  
 corpuscles wandering..... 106  
 " fixed..... 106  
 fibrous reticulated.....107, 159  
 fatty..... 118  
 subperitoneal.....106, 117, 119, 172, 214  
 spaces..... 146  
 adipose..... 159  
 connective..... 218  
 Turpin..... 27  
 Tod.....30, 131  
 Tunica Vaginalis..... 33  
 Trachea..... 37  
 Trauma..... 77  
 Tourneau..... 82, 85, 99, 125, 246  
 Toldt..... 85, 159  
 Thoracic cavity..... 121  
 Tendinous bundles..... 126  
 Tendon..... 143  
 Treitz..... 170  
 Thoma.....187, 355  
 Tube..... 212  
 Thoracic duct (Rabbit's)..... 339  
 Tubby..... 346  
 Turtle..... 395  
 Tract, alimentary..... 215  
 respiratory..... 215  
 Tears..... 215  
 Tunica intima..... 239  
 media..... 239  
 adventitia..... 239  
 Table of experiments..... 379  
 Technique of Peritoneum.....282 to 392  
 ra.....1, 43  
 Vivisection.....2, 9  
 Von Recklinghausen..5, 18, 37, 76, 82, 125, 210  
 Vater..... 5  
 Valentine.....10, 26, 37, 108  
 Virchow.....26, 27, 28, 76, 210  
 Vacuolation.....98, 208  
 Veins, omphalo-mesenteric..... 120  
 subclavian.....223, 224



Veins, umbilical.....	120	Wesling.....	
Valves, lymph.....	141, 216	Waldeyer.....	40
Vasa inferentia.....	156	Wegner, Georg.....	85, 282, 337, 378
efferentia.....	156	Wharton's Jelly.....	116
Valved lymphatic channels.....	204	Wadd.....	132
Vital processes.....	209	Waller.....	182
Vater-Pacinian Corpuscle.....	272	Walls, lymph channel.....	249
Veins (innominate).....	359	Zarvitski's experiments.....	374

## BIBLIOGRAPHY





## BIBLIOGRAPHY OF THE PERITONEUM.

*This Bibliography of the Peritoneum is the most extensive and complete of any that has thus far been published. It is collected from all sources of medical literature. The two chief sources were the catalogue of the Surgeon General's office and the "Index Medicus," besides my own private collection extending over many years. It has been the aim to make the Bibliography full and extensive, and as complete as possible for "Vol. I;" also to perfect it by additions to every "Volume" subsequently issued. All errors will be gladly corrected. The author requests readers to send him the title and name of the writer of any article on the Peritoneum, not here inserted, and he will cheerfully add it to the Bibliography in the volumes which are to be published in the near future.*

BYRON ROBINSON.

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